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THE DEVELOPMENT OF THE FRUIT OF MACADAMIA TERNIFOLIA [1]

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INTRODUCTION

During the course of various investigations in progress at the Hawaii Agricultural Experiment Station on growth and reproduction in *Macadamia ternifolia* F. Muell., close observations were made on the structure of the fruit. As a result of these observations, considerable doubt arose as to the accuracy of the generally accepted classification of the fruit as a drupe and of the shell of the nut as an endocarp or putamen. Von Mueller (*6, v. 6, p. 191*),[3] who named the species, describes the fruit as possessing a horny pericarp with a rather swarthy exterior and a very smooth yellow and date-brown interior, the testa being thin and membranous. Engler (*3*) defines the fruit as a drupe with a fleshy external layer and a thicker, harder inner layer. Bailey (*1*) describes the fruit as having a two-valved leathery exocarp, the endocarp being smooth and shining, thick, and very hard. According to Bentham and Hooker (*2, v. 3, p. 178*), it is a subglobose, indehiscent drupe with a fleshy pericarp and a thick, hard endocarp.

The present paper presents the results of an investigation of the anatomy of the fruit and its parts. The study was nearing completion when an article by Francis (*4*) was brought to the writers' attention. Francis (*4, p. 43*) makes the statement: "A considerable amount of confusion exists in the descriptions of the fruit in systematic, botanical literature." He advances evidence in support of the fact that the nut is truly a seed and the fruit in which it is contained is not a drupe but a follicle. According to Francis, the shell of the nut is not endocarp but is the combined testa and tegmen. The present writers' investigations, on the other hand, show that the shell is made up of testa alone and that the inner integument does not develop into a seed coat. Whereas Francis based his studies mostly upon the mature fruit, the writers' observations covered the development of the fruit from the early flower-bud stages to maturity.

By reporting this work and reviewing the Australian reference, it is hoped that attention will be focused on the inaccuracies of current terminology and that the correct terms may be put into scientific use. While it is likely that in common usage and in the trade the macadamia will always be called a nut, it is important that the investigator have an accurate conception of the material that he is handling.

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[2] The writers take pleasure in acknowledging their indebtedness to Dr. A. J. Eames, of Cornell University, for his helpful discussion and valuable criticism.
[3] Italic numbers in parentheses refer to Literature Cited, p. 406.

MATERIALS AND METHODS

The macadamia is represented in Hawaii by *Macadamia ternifolia* F. Muell. and its botanical variety *integrifolia* (Maiden and Betche) Maiden (*5, v. 1, p. 217*). The differences between the species and its variety are chiefly in size of tree, degree of pubescence, color of flower, shape and size of leaf, and surface of shell. Because these characters have no bearing upon the fundamental structure of the fruit, the materials used in this study were taken from mature seedling trees of the variety *integrifolia*.

The material was killed and fixed in a formalin-alcohol-acetic-acid solution (3 ml. of 40-percent formalin, 90 ml. of 50-percent alcohol and 7 ml. of glacial acetic acid). The long, thick-walled hairs on the pistil and the sclerenchymatous tissues in the fruits necessitated treatment with 48-percent hydrofluoric acid over a period of 3 to 4 weeks for the former, and 5 months or longer for the latter. After a thorough washing in running tap water, the material was processed through Randolph's (*7*) *n*-butyl alcohol series and embedded in paraffin with a melting point of 56° to 58° C. Sections for the flower studies were cut at 10 to 12 μ; for the fruit studies, at 10 to 15 μ.

A 0.3-percent solution of Delafield's haematoxylin in 50-percent alcohol proved to be the most satisfactory stain. The sections were stained for 30 minutes, followed by destaining in a dilute solution of hydrochloric acid for 5 to 10 minutes. Other staining combinations were generally unsatisfactory because of the retention of the stains in many of the cells of certain tissues.

Francis (*4*) performed a number of microchemical tests upon the contents of the heavily staining cells and concluded that the substance is probably a tannin or one of its derivatives, possibly phlobaphene. These contents in all cases give typical tannin reactions with both a ferric chloride and a 1-percent chromic-acid solution. In untreated sections, the cells appear to be filled with a dense, apparently colloidal substance varying from reddish brown to yellow. These cells are evident in the earliest stages of bud development and increase in number as the development of the tissues proceeds. Aside from Francis' suggestion that the substance may be phlobaphene, no attempt has been made to analyze it, nor does there seem to be any satisfactory method of removing it from the cells. As a result, the cells are rendered opaque under the microscope even when the sections have been cut very thin.

In addition to the microchemical tests upon the tannin deposits, Francis made two tests on the green pericarp tissue for the presence of a cyanogen. The first test with Guignard's sodium picrate paper gave a fairly positive reaction; the second with a 0.3-percent aqueous solution of mercurous nitrate gave a deposit of metallic mercury in the parenchyma cells, indicating the presence of a cyanogenetic glucoside or a labile compound.

The cell walls of the exocarp and testa of the fruit assume an intense reddish-violet color upon treatment of sections with phloroglucin and a red color when tested with Maule's reagent (*8*). These reactions indicate the presence of lignin. The lumina of cells in these tissues contain dark-colored deposits of a substance which gives a positive tannin reaction with ferric chloride.

OBSERVATIONS

DESCRIPTION OF THE FLOWER

The flowers of *Macadamia ternifolia* are small, about 12 mm. long at anthesis, perfect and apetalous (fig. 1, *B*), but having four petaloid sepals. They are borne in groups of three or four on pedicels about 3 mm. in length, along the rachis of a spikelike raceme. The pistil is superior, and the stamens are perigynous, being affixed with short filaments to the throat of the floral envelope. The pistil is surrounded at its base by an irregular-margined, glabrous disk (fig. 1, *C*), and it is composed of a single carpellate ovary that is densely pubescent and a style that is glabrous. The ovary contains two suspended, sessile, orthotropous ovules on the margins of its single ventral suture. Before the perianth exposes the anthers, it splits

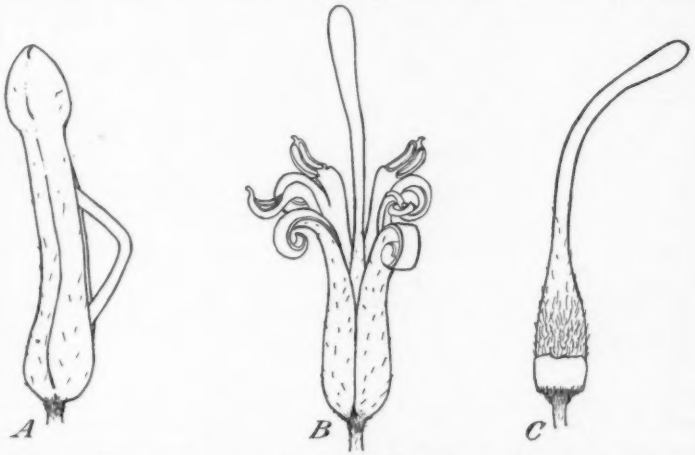


FIGURE 1.—Macadamia flower: *A*, The mature bud showing the perianth splitting to release the style; *B*, the flower in anthesis showing perianth, stamens, and style; *C*, the pistil with perianth removed to show the pubescent ovary, the pubescent style, and the glabrous disk.

along one side exposing the club-shaped style and its small stigma (fig. 1, *A*).

Transverse sections of the flower bud show the arrangement of the perianth parts to be valvate, the edges of the four sections joining one another by means of interlocking epidermal cells (pl. 1, *A*). The perianth envelope is regular and minutely pubescent. At anthesis its reflex points spread open to release the four two-celled anthers of the perigynous stamens.

A study of the vascular system of the flower shows that three traces, one dorsal and two ventral, branch from separate gaps in the stele of the receptacle into each of the four perianth parts (fig. 2, *C*, *D*). This three-trace condition indicates the sepal nature of the perianth. The single bundle of the stamen passes off directly after the dorsal bundle of the perianth part, to which it is adnate. The two bundles run parallel to the point at which the stamen is no

longer attached to the perianth. Vascular supply to the disk precedes that to the carpel and consists of several small strands containing very little xylem. The function of this disk is probably glandular. The carpel has five or seven main bundles (fig. 2, *A*, *B*). The exact number of traces derived from the stele is difficult to determine because the bundles all arise at about the same level and branch readily after their departure. The extra traces lie between the dorsal and ventrals on either side. The vascular system of the ovules is derived from the ventral bundles (fig. 2, *B*).

INITIAL STAGES IN FRUIT DEVELOPMENT

Ovule formation is evident in buds about 1.0 to 1.5 mm. in length (pl. 1, *A*). A well-defined suture can easily be traced throughout the entire length of the short pistil. On either margin of the suture a mass of meristematic tissue protrudes into the ovarian cavity.

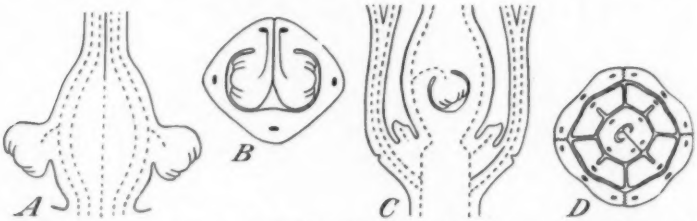
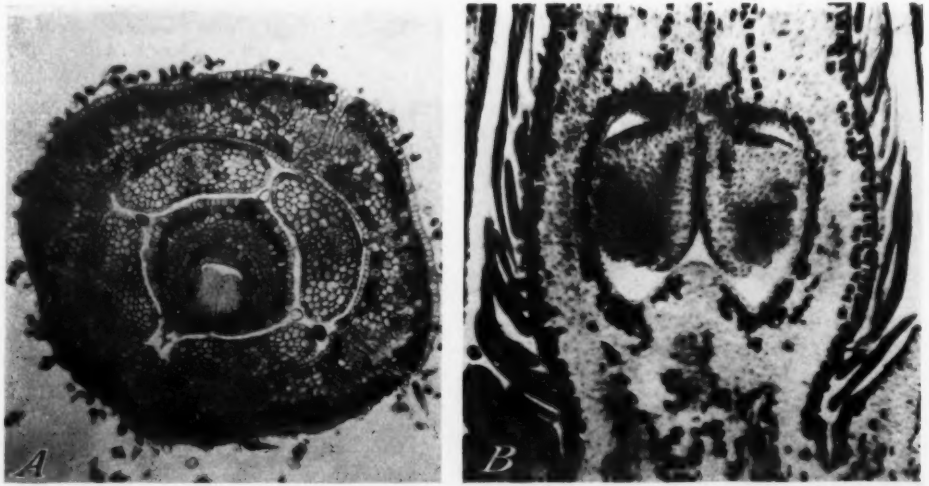


FIGURE 2.—Vascular system of *Macadamia ternifolia* flower: *A*, Carpel split ventrally and spread open, showing trace number and venation; *B*, transverse section; *C*, median longitudinal section of base of flower showing course of traces to floral organs—dorsal and only one ventral trace shown in carpel; *D*, transverse section.

These masses of ovule tissue increase in size by rapid cell division until an average size of about 86 by 122μ is reached, when the inner and outer integuments appear as two small folds of tissue protruding from the base of the developing ovule (pl. 1, *B*). All of the cells of the ovule are typically meristematic at this stage, having diameters essentially alike, thin cellulose walls, large nuclei, and small vacuoles. The outer integument is shorter and thicker than the inner which, at this stage, is two or three layers thick, tapering off to a single layer as it approaches the micropylar end. The ovules are crowded against the ovary wall at the top and sides so that both integuments remain closely associated with the nucellus up to the time that megasporogenesis begins.

By the time the flower bud has fully developed, megasporogenesis has been completed, followed by the elongation of the nucellus and the development of an embryo sac. The integuments have also elongated and now invest almost the entire nucellus (pl. 2, *A*). Deposits of tannin, or tannin derivatives, become abundant in the two outermost layers of the outer integument; the nuclei of the cells in these layers persist for a while after they have become filled but later disintegrate, leaving only spaces or lumina.



Macadamia ternifolia: A, Transverse section through a bud 1.5 mm. long showing the mass of meristem protruding into the ovarian cavity initiating ovule formation. Note interlocking of epidermal cells between perianth parts. *B,* Dorsal view of longitudinal section through the ovary of a bud 4 mm. long showing the two pendulous ovules with the outer and inner integuments being initiated. *A, B,* × 100.

Macadamia ternifolia: *A*, Longitudinal section through ovary just prior to fertilization, showing the position of the integuments. *B*, Longitudinal section of portion of young fruit 2 mm. in diameter showing increase in size of the testa and the inner integument with its opaque inner epidermis. Dark-colored layer of pericarp is endocarp, light-colored portion in upper left corner is exocarp; the light central area is nucellus tissue surrounding the embryo sac in which endospermic nuclei are present. *C*, Transverse section showing a normal and an aborting seed. Note prominence of inner epidermis of ovary wall. *A–C* × 100.

INTERMEDIATE STAGES IN FRUIT DEVELOPMENT

After fertilization, the perianth and disk of the flower drop off, leaving only the pistil on the pedicel. As the ovary develops, the long style gradually dries back and falls off, only the remnant of the stylar projection remaining on the fully developed fruit.

A longitudinal section of the ovary at approximately the time of fertilization shows that the carpel wall is made up of a thick layer of parenchymatous and vascular tissues with an outer epidermis from which arise many thick-walled hairs and an inner epidermis of small active cells which lines the ovarian cavity (pl. 2, *A*). This inner epidermis is conspicuous in the developing fruit (pl. 2, *B*, *C*) but disintegrates before maturity is reached. The suture is prominent as a small furrow extending along one side from the apex to within a few millimeters of the base. As seed development goes on within, the ovary wall increases in thickness and differentiates into two layers, the exocarp and the endocarp (pl. 2, *B*). The endocarp is composed of parenchyma cells only, but the immature exocarp is composed of both parenchyma cells and primary vascular elements.

The outer and inner integuments expand slightly to allow for development of the embryo tissues. The inner integument, three cell layers thick, increases in length to encompass the micropylar portion of the nucellus and protrudes into the micropyle (pl. 2, *B*, and Fig. 3, *B*). Further differentiation in this tissue is limited to the inner epidermal layer which becomes opaque from impregnation with what appears to be tannin substances. The tissue remains intact through the early stages of maturity and in later stages it is present as a layer of disintegrated tissue adhering to the white portion of the inner surface of the shell or testa (fig. 3, *E*). The mature embryos tend more or less to cling to the white enamel portions of shell or outer seed coat. This adherence may be due in part to the presence of the vestigial inner seed coat.

The outer integument, which should now be referred to as the seed coat or testa, does not appear to be active at this stage; but when the embryo is differentiating to form cotyledons and the plumule-radicle axis the cells of the testa multiply and enlarge, and the tissue completely surrounds the embryo. Development is particularly active in the chalazal region and accounts for the thicker layer of shell at this point. The meristematic cells forming the inner epidermis of the testa divide to form a tissue three to five cells thick. These cells form the white enamel portion of the inner layer of the shell (fig. 3, *D*, *E*).

Endosperm development is rapid during a period of from 4 to 8 weeks after fertilization. In its early stages the endosperm is multinucleate, the nuclei and cytoplasm being limited to a peripheral layer around a central vacuole. By the end of approximately 8 weeks, cell walls have formed and the gelatinous endosperm occupies all but a very small portion of the embryo sac. The nucellus is not completely absorbed, several layers of it persisting in the developing seed up to the last stages prior to maturity (fig. 3, *D*). Although at first the development of the embryo is more gradual than that of the endosperm, by the end of approximately 20 weeks the cotyledons completely replace the endosperm-tissue.

The two ovules appear to be alike in every respect until the time of fertilization, but usually only one of them develops in the fruit (fig. 4). The causes of the abortion of one are still unknown, although abortion appears to be a varietal characteristic. The aborted structure may be found adhering to the endocarp or, in various stages of development, clinging to the normal seed.

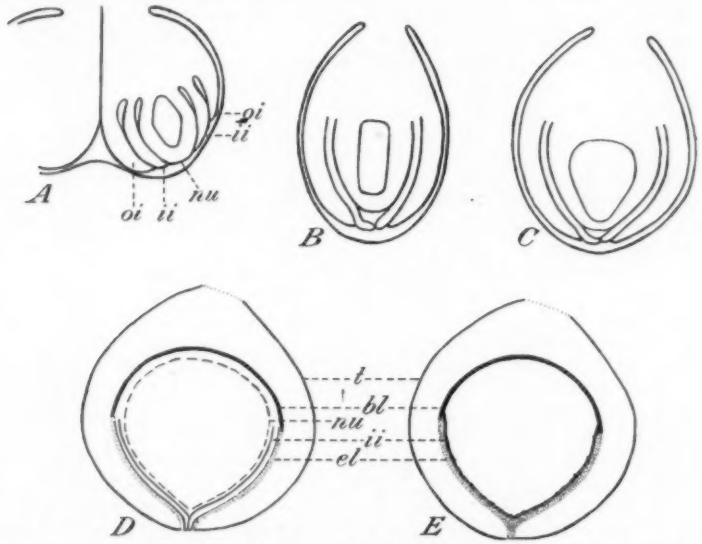


FIGURE 3.—*Macadamia ternifolia:* Longitudinal diagrams showing development of outer integument and changes in inner integument and nucellus tissue from bud stage to mature seed: *A*, Bud stage showing positions of two ovules, outer integument, inner integument, and nucellus tissue; *B*, fertilized ovule showing inner integument protruding into the micropyle; *C*, intermediate stage with nucellus being absorbed by developing embryo; *D*, shell of seed before it hardens showing remnants of nucellus, the white enamel layer developing from inner epidermis of testa and the brown layer; *E*, hardened shell with disintegrated inner integument clinging to white enamel portion. *oi*, Outer integument; *ii*, inner integument; *nu*, nucellus; *t*, testa; *bl*, brown layer; *el*, enamel layer. *A*, *B*, × 100; *C* × 90; *D*, *E*, × 22.

THE MATURE FRUIT

The entire mature fruit is a follicle, globose, and slightly oblique in shape, which, when ripe, dehisces along the ventral suture (fig. 4, *C*). The seeds are globular (fig. 4, *D*) except when both ovules have developed, in which case they are hemispherical, with one located in each valve of the dehiscing pericarp.

The kernel of the seed is composed of two semiglobose cotyledons enclosing a small, subglobose plumule-radicle axis.

THE PERICARP

The dehiscent pericarp consists of two distinct layers of tissue, a fibrous exocarp with a dark-green, fairly smooth exterior and a softer, thinner endocarp. The exocarp has an epidermis overlying a thin layer of chlorophyll-bearing cells, the main body being made up

of parenchyma tissue in which are embedded numerous freely branching vascular bundles. The endocarp is devoid of bundles, and its parenchyma cells are filled with dark tannin-like substances (pl. 2, *B*).

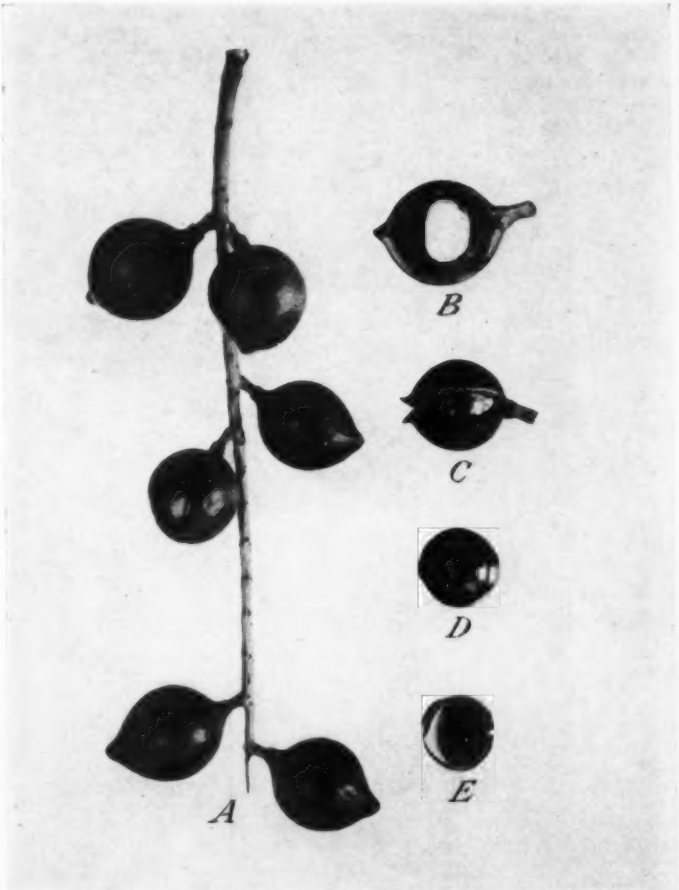


FIGURE 4.—*Macadamia ternifolia: A*, Mature green fruits on rachis; *B*, mature green fruit split in half showing the embryo and its enclosing shell within the pericarp; *C*, split pericarp with mature seed; *D*, seed; *E*, empty half shall showing the cream-colored portion of the inner surface (micropylar end at left) and dark-brown portion (chalazal end at right). × ½.

THE SEED COAT

The so-called nut contained in the pericarp is a true seed having a seed coat, a hilum, and a micropyle (fig. 4, *D*, *E*). The seed coat has developed from the outer integument into the hard, bony shell, generally mistaken for an endocarp.

The testa, as mentioned above, is made up of two distinct layers. The outer, thicker portion is a very hard sclerenchyma tissue of fiber and stone cells. The elongated fiber cells predominate and occur in strands or sheets extending both longitudinally and radially. The stone cells vary considerably in size and shape and contribute to the rigidity of the tissue by forking and interlocking (pl. 3, *B*). The walls of both types of cells are highly lignified and abundantly pitted. Fine vascular bundles with spirally thickened elements are also seen scattered throughout the tissue.

The inner layer of the shell is about one-fifteenth as thick as the outer layer. About one-half of the shell interior, toward the hilar

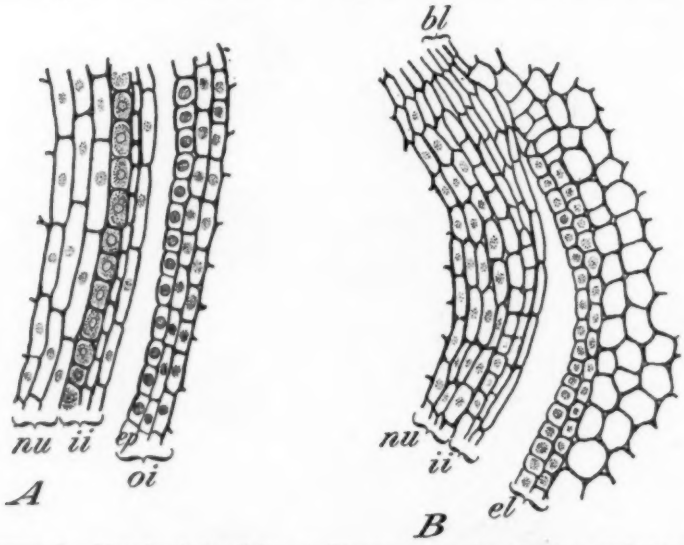
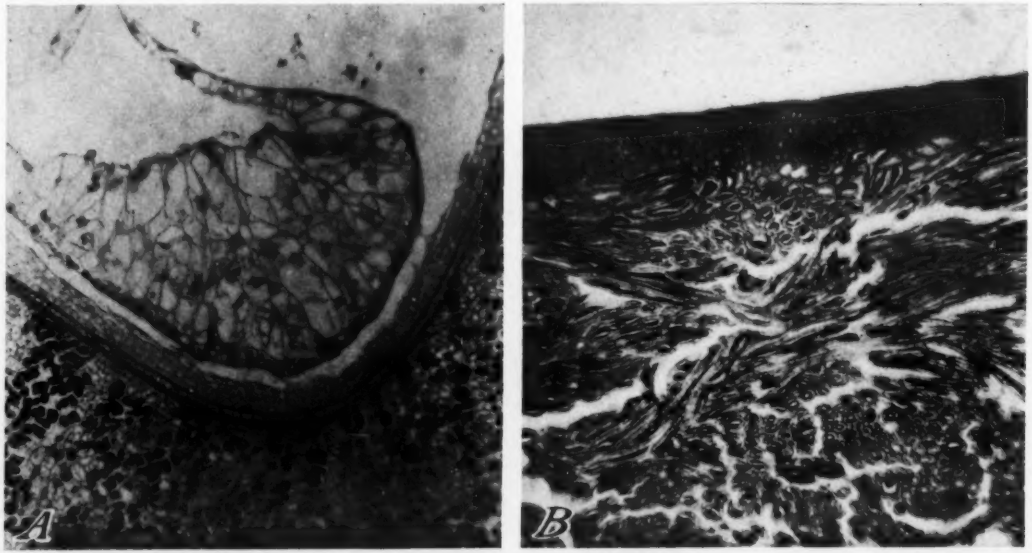


FIGURE 5.—*Macadamia ternifolia: A*, Diagram of section of young seed showing cells of nucellus, inner integument, and inner layers of outer integument. The heavily shaded layer is filled with tanninlike deposits. *B*, Section from older seed through region where the integuments come together showing development of enamel layer from epidermal cells in outer integument, and cells of brown layer. *nu*, Nucellus; *ep*, epidermis; *ii*, inner integument; *oi*, outer integument; *el*, enamel layer; *bl*, brown layer. × 500.

end, is shiny and dark brown while the half toward the micropylar end is enamellike and cream-colored (fig. 4, *E*). Francis (*4*) believed this entire inner layer to be tegmen developed from the inner integument, but the sections made by the writers show that it is a part of the testa. Differentiation of the inner layer begins at that point in the chalazal region where the outer and inner integuments originate (fig. 5, *B*). The brown section, which develops around the embryo at its apical or broader portion, is composed of somewhat flattened and compacted cells, filled with a brown deposit similar to that which fills the cells in the outer layer of the testa. The cream-colored section covering the lower or basal portion of the embryo is composed of cells derived from the inner epidermis of the outer integument (fig. 5, *A*).



Macadamia ternifolia: A, Longitudinal section of young seed showing testa, nucellus, and endosperm tissue. *B,* Section of a mature shell showing the arrangement of the stone cells of the testa and the dense polygonal cells of the white enamel layer. Rupture of tissue is caused by cutting. ×100.

These cells occupy a position parallel to that of the undeveloped inner integument which lies within it (fig. 3, *E*). Francis made a very complete and detailed study of the compact polygonal cells making up the enamel portion. The walls seem to be unstratified and without abundant pits. The granular material contained in the cells is lignocellulose. In prepared sections of this tissue, each cell has a small, angular, clear space, which, according to Francis (*4, p. 47*), "indicates a position in the cell occupied by a crystal of calcium oxalate, which appears to have been dissolved either by the treatment involved in the preparation and mounting or by the natural processes of the maturing seed."

DISCUSSION

A drupe falls under the general classification of simple fleshy fruits. It is derived from a single carpel and is usually one-seeded. There may be three distinct layers in the ripened pericarp, in which case there is a thin exocarp forming the skin, a fleshy mesocarp, and a stony endocarp; or, as is more common, there may be only two layers, a fleshy exocarp and a stony endocarp. A nut is usually defined as a hard, dry, indehiscent, one-seeded and one-celled bony fruit even if it represents a compound ovary. A follicle on the other hand is a dehiscent, unicarpellate fruit which splits along one suture at maturity and contains one or more seeds. By definition, therefore, the macadamia fruit is not a drupe but is a follicle, and the "nut" is not truly a nut but is the seed contained in the follicle.

While it hardly seems likely that in common usage and in the trade the macadamia "nut" will ever be called anything else, it should be borne in mind that it is properly a seed and that the shell is not putamen but is a well-developed testa. It may be seen how the unusually hard, thick shell of the seed could be mistaken for the stone of a drupe by the early systematic botanists and how the name of nut could be applied to it in common usage.

The development of the outer integument into a thick, hard testa is not peculiar to the macadamia alone, for the hard shell of the Brazil nut (*Bertholletia excelsa*) also is a seed coat and not a pericarp tissue.

The brown smooth portion and white enamel layer of the shell were interpreted by Francis as being a tegmen developed from the inner integument. The observations of the writers lead them to believe that these portions are actually a part of the testa (outer integument). The enamel layer is developed from the cells of the inner epidermis of the outer integument. The cells of the brown layer develop from the seed-coat tissue at the base of the ovule and form a line which separates the developing kernel from the testa.

SUMMARY

Anatomical investigations indicate that the fruit of *Macadamia ternifolia* F. Muell. is a follicle and not a drupe. The pericarp is dehiscent along a single suture and is made up of a thick, leathery exocarp and a thin, soft endocarp. The so-called nut is a true seed having one seed coat that develops from the outer integument of the ovule, a hilum, and a micropyle.

The shell of the nut is the testa. The brown and white layers lining the inside of the shell are developed from the testa.

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SOIL CHARACTERISTICS, TOPOGRAPHY, AND LESSER VEGETATION IN RELATION TO SITE QUALITY OF SECOND-GROWTH OAK STANDS IN CONNECTICUT[1]

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INTRODUCTION

Approximately 56 percent of the area of Connecticut is in forest and brushland. While the species growing within its borders are many, the predominant forest type is oak. Because of the importance of oak in Connecticut forests, it was deemed advisable to ascertain the relationship, if any, existing between growth of oaks (expressed as site quality or site index, and measured in terms of the height growth of dominant trees) and various topographic, soil, and vegetative factors.[3] This study is primarily an extension of investigations begun with red pine and published in 1931 (*10*).[4]

As a recent publication by Aaltonen (*1*) includes an excellent review of the literature, it is unnecessary to discuss it in detail here. Taken as a whole, the findings are conspicuously dissimilar. Some investigators have observed some correlation between site quality and nutrient content of the soil; others have found none. In some cases the biological properties of the soil were found to be of marked significance. Practically all agree on the importance of soil moisture, and in many cases site quality varies with the proportion of silt and clay in the soil. In the case of sandy soils in the vicinity of Eberswalde, Germany, the presence of an underlying marl or clay layer within reaching distance of the tree roots frequently is the chief factor in controlling stand composition and yield, according to Ganssen and his coworkers, and Wiedermann, as reported by Aaltonen (*1*). In his English summary Aaltonen states:

> Determination of the productivity of soil on the basis of its properties is one of the most important objects of forest soil research. At the same time, it is one of its most difficult problems, for it seems that the productivity seldom depends upon a single factor or even a few factors, but is usually a result of the combined actions of several factors.

Burns (*3*) was thinking along the same line when he wrote, "The amount of increment and the width of the annual ring in a tree's growth show its response to the physiological summation of all the factors of the site." Turner (*19*, *20*), working in Arkansas, states that "soil features influencing available water seem to be more influential than any others in determining the rate of growth of pine trees" (*19*, *p. 11*). He found no correlation between occurrence of growth

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[2] Acknowledgment is made to Henry W. Hicock, assistant forester, Connecticut Agricultural Experiment Station, for advice and assistance, especially in the calculation of the site indices.
[3] The field work was done in 1930, principally by Henry Bull, formerly assistant in forestry, Connecticut Agricultural Experiment Station.
[4] Italic numbers in parentheses refer to Literature Cited, p. 427.

and any single soil factor; but he did find a correlation with certain "site-soil complexes."

One may sift from the many papers on this subject at least two fairly well established conclusions that are generally applicable in the principal forest regions of Europe and the United States: (1) Available moisture is usually the factor of prime importance in all but heavy or poorly drained soils; and (2) lime is usually second in importance. Climate and soil vary so greatly that each forest district and in some cases each individual stand presents its own peculiar problems which require separate investigation and solution.

With respect to the oak stands in Connecticut, Frothingham in 1910 (*6*) made a study of growth, uses of lumber, and methods of management of second-growth chestnut, oak, and mixed hardwoods. He did not study the soils but merely stated that "soil conditions are largely responsible not only for differences in type but also for variations in the rate of growth within the same type." At that time chestnut occupied the better soils, and the oaks, especially black, scarlet, and chestnut oak, were confined largely to dry upper slopes and ridge tops. Since the disappearance of the chestnut its place has been taken principally by red oak. As Frothingham's yield data have been largely superseded by the recent work of Schnur (*16*), no further reference to the former will be made here.

In 1926 and 1927 Hicock and others (*10*) attempted to ascertain the relationship between soil type and stand composition in four State forest tracts. The results were largely negative, principally for the following reasons: Probable biological equivalence of two or more soil types; generally favorable climate; and effect of fire and other factors brought about mainly through the intervention of man.

METHODS

SELECTION OF PLOTS

The 76 stations (hereinafter called plots although they were of no definite size or shape and were not marked off) used in this study were taken wherever there was an even-aged stand in which the great majority (80 percent or more) of the dominant trees were oaks. Stands were considered even-aged if the dominants did not vary by more than 10 years and were reasonably uniform in height. On at least 80 percent of the plots the range of ages of dominants was not more than 4 to 5 years. Five species of oaks are well represented, namely—white (*Quercus alba* L.), red (*Q. borealis maxima* (Marshall) Ashe), black (*Q. velutina* La M.), scarlet (*Q. coccinea* Muench.), and chestnut (*Q. montana* Willd.). Two other species—swamp white (*Q. bicolor* Willd.) and pin oak (*Q. palustris* Muench.)—were present to a limited extent and some data on these are included. The number of trees measured on each plot averaged about 20, although it varied from less than 10 to about 50.

FIELD MEASUREMENTS AND OBSERVATIONS

Measurements in the field included height, diameter at breast height (d. b. h.), and age of dominant and codominant oaks, separately for each species. Intermediate and ground-cover vegetation were listed and estimated as to density and uniformity of distribution. Careful notes were taken on the topography and related environmental fea-

tures, character of the forest floor,[5] and soil description. In addition, the mineral soil to a depth of about 5 inches was sampled for study in the laboratory.

CALCULATION OF SITE INDICES

The height curves and formulae of Schnur (*16*) (obtained through the Northeastern Forest Experiment Station prior to publication) were used as a basis for the calculation of the site indices. In determining total age, 3 years was added to the age at breast height. Schnur, on the other hand, used a correction factor of only 2 years, although this was not known by the writer until the appearance of Schnur's bulletin. The data have not been revised to correspond with Schnur's in this respect, for the following reasons: (1) Many trees in this study came from seedlings that grow slowly, while apparently most of those represented in the Forest Service study were sprouts; (2) on the whole, growing conditions in Connecticut are not quite so favorable as those in most of the areas covered by the Forest Service study; and (3) the differences in site index resulting from the 1-year difference in estimated age are quite small (average difference in the first 10 plots recorded in this study was 0.8 foot).

The figures for the height of the average tree (average basal area) used in the calculations were obtained by plotting the measured heights against the measured diameter at breast height, and then using the diameter at breast height by basal area to determine the height.

The principal equation used in calcuating site index was:

$$I \text{ (site index)} = 62.7 + 8.37 \frac{H - Ha}{\sigma a}$$

where

H = average dominant height of the stand in question
Ha = average height at any age a
σa = standard deviation of height about the average at any age a

A plotting of these 76 plots on the Forest Service set of curves indicates that the trend of an average curve representing all of these plots does not follow very closely the trends of the Forest Service curves. Since the plots were taken where suitable stands could be found and do not necessarily constitute a good random sample, this lack of agreement is not significant. However, since the Forest Service found no significant differences between oaks from a region reaching from Missouri to Maryland and from Tennessee to Michigan, it is reasonable to believe that Connecticut oaks would not be an exception in this respect. Therefore, for present purposes we can assume applicability to Connecticut conditions.

SOIL STUDIES

The following laboratory analyses were made on the soil samples collected in this study: Total nitrogen, loss on ignition, reaction (pH), exchangeable hydrogen,[6] total base capacity,[6] percent saturation,[6] conductivity,[7] moisture equivalent, total colloids,[8] and soluble nutrients by quick test.[9]

[5] Forest floor = the whole organic debris, including litter, F and H layers.
[6] Determined by the $Ba(C_2H_3O_2)_2 - NH_4Cl$ method of Pierre and Scarseth (*15*).
[7] Electrical conductivity of a water suspension.
[8] Determined by the hydrometer method of Bouyoucos (*2*).
[9] Morgan's Universal soil test.

DESCRIPTION OF THE REGION

Connecticut lies at the northeastern edge of the upland oak region, with some intermingling of the whitepine types in the northern part of the State. The forest associations generally recognized (*14*) are: Red cedar-gray birch association, 20 percent (of the total forested area); hardwood association, 70 percent; hemlock-hardwood association, 5 percent; and swamp association, 5 percent. In the hardwood association the oaks predominate.

Climatically, Connecticut is characterized by relatively long, cold winters and rather short, humid summers. The mean annual temperature is 47° to 49° F.; mean maximum in winter, 31° to 38°; mean minimum in winter, 15° to 20°; length of growing season, 150 to170 days; mean annual precipitation, 44 to 46 inches, rather evenly distributed throughout the year. In table 1 are given the temperature and rainfall data by months.

TABLE 1.—*Temperature and rainfall data for Connecticut* [1] *by months and the annual average or total*

Month	Temperature			Mean precipitation	Month	Temperature			Mean precipitation
	Mean	Mean maximum	Mean minimum			Mean	Mean maximum	Mean minimum	
	° *F.*	° *F.*	° *F.*	*Inches*		° *F.*	° *F.*	° *F.*	*Inches*
January	26.6	35.4	18.1	3.84	August	68.6	78.7	58.3	4.27
February	26.1	35.2	17.0	3.77	September	62.3	72.9	52.0	3.75
March	35.0	44.7	26.2	4.11	October	51.6	62.6	41.5	3.63
April	45.9	56.5	35.6	3.69	November	40.3	49.5	31.3	3.62
May	57.1	68.3	45.9	3.66	December	29.6	38.0	21.3	3.84
June	65.6	76.4	54.6	3.31					
July	70.6	81.0	60.4	4.17	Average or total	48.3	58.3	38.5	45.65

[1] Average means from 7 stations well distributed over the State as follows: Colchester, Cream Hill (Cornwall), Storrs, New Haven, Southington, Voluntown, Waterbury. All records for periods of 30 years or more.

Since we are attempting to apply to Connecticut site-index curves obtained by the Forest Service in the region extending from Maryland to Missouri and from Michigan to Tennessee, it is well to compare climatic conditions. This is done in table 2 through the use of Jenny's data (*12*). (A low rain factor and a high *NS* quotient indicate favorable growing conditions.) Here we see that in comparison with the other areas, Connecticut has a slightly higher rainfall but a lower mean temperature, resulting in a decidedly higher rain factor. Relative humidity is practically identical, but the *NS* quotient is higher. The net result is a somewhat less favorable growing condition than the average of the other areas.

Elevations in Connecticut range from sea level to a maximum of 2,355 feet, with the greater portion of the land lying between 100 and 1,500 feet.

The soils of Connecticut belong to the Brown Podzolic group which is found only in the Northeastern States south of the Podzol area of New England.

Essentially, the Brown Podzolic soil is an imperfectly developed Podzol having, in timbered areas, an organic mat on the surface and a very thin gray leached horizon just below it—usually less than an inch thick. The B horizon is largely

yellowish brown in color and has only the beginnings of a dark-brown orterde just below the gray A horizon. The total depth of the solum is usually less than 30 inches although it exceeds that depth in places (*21*).

Fine sandy loams predominate in the forested areas and are prevailingly acidic, being derived principally from granitic and dioritic gneisses and schists and, in the central lowlands, from red sandstones and shales. Soils from basaltic traprock constitute a very small proportion of the whole.

TABLE 2.—*Comparison of climatic factors in Connecticut and certain other States*
[Data taken from Jenny (*18*)]

Places compared	Precipitation		Temperature		Relative humidity	Rain factor $\frac{\text{Precipitation, mm.}}{\text{Temperature, ° C.}}$	N.-S. quotient [1]
	Millimeters	*Inches*	*° C.*	*° F.*	*Percent*		
Connecticut:							
Hartford	1,175	46.26	9.78	49.6	71.5	120	457
New Haven	1,163	45.78	9.94	49.9	73.0	117	472
Average	1,169	46.02	9.86	49.75	72.25	118.5	464.5
Average of 33 stations in 10 States: [2]	1,002	39.44	11.84	53.31	72.50	84.6	357

[1] N.-S. (N.-S. = Niederschlagsmenge–Sättigungsdefizit) quotient = $\frac{\text{Precipitation}}{\text{Absolute saturation deficiency of the air.}}$

[2] Illinois, Indiana, Kentucky, Maryland, Michigan (south), Missouri, Ohio, Pennsylvania, Tennessee, and West Virginia.

Forest humus types vary from excellent mulls through granular mors [10] to extreme fibrous mors (*9*). Greasy mor is not common and, in general, is found only under hemlock. By far the prevailing type under oak and mixed hardwood stands is a fairly good form of granular mor.

As is true elsewhere, the best areas are used for agriculture so that most of the land in forest or brush falls into one or more of the following categories: Rocky, shallow, steep, low fertility, excessively sandy or gravelly, swampy.

The history of land utilization is approximately as follows (*5*): Until about 1700 practically all of southern New England was forested with a large variety of hardwood species, some hemlock, and probably a little white pine in the northern portion. Then with the marked increase in settlement by the white man, much of the timber was cut and the land used for farming—including a great deal of rough and stony land that should never have been cleared. The maximum amount of land used for agriculture reached its peak between 1830 and 1850, after which, with the opening of the better farm land of the West, there began a gradual diminution of farming and slow reversion of the land to brush and forest.[11] This process has continued to the present day.

[10] By "mor" is meant a type of forest humus in which the organic matter is practically unmixed with the mineral soil and is usually more or less matted or compacted.

[11] The normal succession (*14*) on the poorer sites is: Shrubs (such as *Myrica* and *Rhus*) →red cedar-gray birch→inferior hardwoods (dogwood, hophornbeam, blue beech, red maple, shadbush, chokecherry, sassafras, butternut, pignut hickory, bitternut hickory, and large-toothed aspen) →better hardwoods (red oak, scarlet oak, white oak, chestnut oak, black oak, white ash, shagbark hickory, mockernut hickory, black birch, paper birch, yellow birch, beech, yellow poplar, black cherry, white elm, sugar maple, basswood)→ hemlock-hardwoods (climax). On the better sites the shrub stage is omitted.

The characteristic practice among forest and wood-lot owners is to clear-cut as soon as the trees are big enough to make cordwood and then allow the land to grow up again to forest in its own way without control or direction.[12] Such practice means that Connecticut is producing not more than a third of the wood that could be produced under intensive, intelligent silviculture (7).

PRESENTATION OF RESULTS

SITE INDICES

The data pertaining to age, height, and diameter at breast height, and site index are summarized in table 3. In table 4 is found the plot distribution by site index and age. Seventy-four percent of the plots have a site index between 50 and 70, and 78 percent lie between the ages 20 and 60. In the case of the Forest Service study, the corresponding values were 77 and 72 percent, respectively.

TABLE 3.—*Averages and ranges of age, height, diameter-breast-high, and site-index data for forest plots studied*

Item	Average	Range	Item	Average	Range
Age.....years..	43.2	12–105	D. b. h. by basal area..inches..	7.75	1.9–14.7
Height.....feet..	53.2	17–81	Average height from curve .....feet..	52.9	16–81
D. b. h.....inches..	7.98	2.2–15.6	Site index.....	59.7	30–83

TABLE 4.—*Plot distribution by site index and age*

Total age (years)	Plots in site-index class—						Total	
	20–39	40–49	50–59	60–69	70–79	80–89		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
10–19.....	.....	1	.....	.....	.....	.....	1	1.3
20–29.....	.....	.....	4	4	3	1	12	15.8
30–39.....	.....	.....	5	6	3	1	15	19.7
40–49.....	--	2	8	9	1	.....	20	26.3
50–59.....	1	4	6	1	.....	.....	12	15.8
60–69.....	.....	1	5	2	.....	.....	8	10.5
70–79.....	.....	1	1	2	.....	.....	4	5.4
80–89.....	1	.....	1	1	.....	.....	3	3.9
90–99.....	.....	.....	.....	.....	.....	.....	.....	.....
100–109.....	.....	.....	1	.....	.....	.....	1	1.3
Total.....	2	9	31	25	7	2	76	.....
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Proportion.....	2.7	11.8	40.8	32.8	9.2	2.7	.....	100.0

When the site indices were segregated by species, the data summarized in table 5 were obtained. The black, red, and scarlet oaks ran somewhat higher in site index than the white and chestnut oaks. This is a little unusual, for white oak generally shows a more rapid growth than either black or scarlet oak. Red oak led in the number of plots. Of the several species investigated, only black oak was found in the site index class above 80.

[12] "On land which has been cut over, burned, grazed, or otherwise severely disturbed, but not to the exclusion of all former forest growth, hardwoods usually control the situation from the outset. As a rule inferior hardwoods predominate at first, later on giving way to hardwoods of the better type. On poor sites, however, the red cedar-gray birch stage may precede the hardwood stage * * *. In general, succession on this class of land is more rapid than on old field land" (14).

TABLE 5.—*Summary of site index by species*

[Number of plots in parentheses]

Site-index class	Black oak	Red oak	Scarlet oak	White oak	Chestnut oak	Swamp oak	Pin oak
30-39		34.6 (1)	37.3 (1)		32.7 (1)		
40-49	48.9 (2)	45.0 (1)	49.1 (1)	46.1 (5)	48.0 (3)		
50-59	57.9 (6)	55.1 (9)	54.9 (8)	56.0 (14)	52.5 (4)	59.0 (1)	
60-69	65.0 (9)	64.9 (16)	64.1 (14)	62.6 (11)	64.2 (4)		61.4 (1)
70-79	71.9 (2)	75.4 (7)	74.8 (4)		70.1 (1)		
80-89	84.1 (2)						
Average (of individual plots)	63.9 (21)	63.0 (34)	61.5 (28)	56.8 (30)	54.9 (13)		

CORRELATION OF SITE INDEX WITH CHARACTERISTICS OF THE UPPER MINERAL SOIL

When we attempt to correlate the site index of all plots with certain qualities of the soil as sampled (upper 5 inches of mineral soil), the results obtained are very conflicting, as the summary in table 6 shows. With total colloids (particles <0.05 mm.) and moisture equivalent, the correlation is negative; with total sands and nitrogen, slightly positive; and with pH value, very slightly positive. In no other case is there any correlation except in the quick tests where Ca, P, K, and NH_3-N shows a positive correlation. However, in these tests, the data are characterized by extreme deviations within each site class which greatly reduces the significance of the means. Quick tests are only roughly quantitative and cannot be expected to reveal fine differences. (It should be mentioned that the quick-test values herein reported cannot be compared with similar data obtained at a later date for the reason that both the procedure and the color scale were changed. The data as given have value only for comparison.)

TABLE 6.—*Summary of relation between site index and soil characteristics in all 76 plots*

Soil characteristic or constituent	Data for site-index class—						Correlation indicated
	20-39	40-49	50-59	60-69	70-79	80-89	
Average site index	33.2	46.4	56.0	64.3	74.3	81.9	
Plots—number	2	9	29	27	7	2	
Total colloids—percent	20.0	17.2	14.4	14.6	12.5	7.5	Inverse.
Total sands—do	44.2	57.4	61.7	58.8	63.3	81.6	Positive.
Moisutre equivalent—do	26.3	23.4	20.5	21.3	19.5	10.3	Inverse.
Total nitrogen—do	.175	.202	.180	.206	.230	.101	Uncertain.
Loss on ignition—do	10.33	11.25	8.97	10.06	7.53	3.73	Do.
Reaction—pH	4.48	4.63	4.69	4.71	4.83	5.02	Positive.
Exchangeable hydrogen [1]—milligram equivalents.	10.20	8.92	8.72	9.34	9.46	4.96	Slightly inverse.
Total base capacity [1]—do	16.65	16.56	15.62	16.76	17.04	8.29	None.
Saturation—percent	38.8	45.8	43.8	43.7	44.4	40.3	Do.
Conductivity	9.53	10.22	10.01	10.10	9.79	7.72	Do.
Soluble nutrients per acre by rapid soil tests:							
Calcium—pounds	10	12	25	26	34	35	Positive.
Phosphorus—do	1.5	3.2	3.4	3.8	3.6	5.5	Do.
Potassium—do	35	35	47	41	64	50	Slightly positive.
Ammonia nitrogen—do	50	58	58	57	75	100	Do.
Aluminum—do	25	24	25	27	29	14	None.
Iron (ferric)—do	55	18	22	13	16	8	Slightly inverse.

[1] Per 100 gm. of soil.

Correlations were attempted between soil characteristics and site index of the individual dominant species, considering all plots. This did not prove any more satisfactory than it had where all species were considered together.

This lack of correlation between site index and soil characteristics may be ascribed to other environmental factors which exert an influence on tree growth greater than does the soil. In any forest, soil is only one factor, albeit an important one, in making up the site. It is quite evident from table 6 that other factors must have been operative in some of the plots. Reference to the field notes revealed that on certain plots—11, to be exact—the effect of the soil was obviously overshadowed by other factors. For example, some of the plots on sandy soils that were unquestionably low in fertility had a high site index because of their close proximity to a lake or stream. This meant that the roots had access to an abundant supply of water which favored growth.

Likewise at the other end of the scale, there were plots exhibiting a low site index although the soil sample did not indicate an inferior soil. In some of these cases the soil consisted of a relatively shallow mantle over bedrock; and frequently there was a dense growth of *Vaccinium* or *Gaylussacia* which offered severe competition to the trees. Some plots were located on extremely rocky areas where there was a tendency for the organic matter to accumulate in the crevices between the rocks, and in such cases the soil sample was of little significance. Rockiness in itself does not lower the site quality for forest trees; rather it may improve conditions by favoring aeration, moisture content, and root penetration. Such soils, however, are extremely difficult to sample with any assurance that the sample is representative of the whole soil mass penetrated by the roots.

The presence of any of the afore-mentioned factors is sufficient reason for excluding such plots from consideration so far as soil studies are concerned. Therefore, in table 7 correlations have been attempted between site index and certain soil qualities on 65 plots, omitting the 11 mentioned above. In this instance the groupings by site index were changed somewhat in order to more nearly equalize the number of plots in the several groups.

TABLE 7.—*Summary of relation between site index and soil characteristics of 65 plots*

Soil characteristic or constituent	Data for site-index class—					Correlation
	40–54	55–59	60–64	65–69	70+	
Average site index	50.7	57.7	61.7	67.4	75.8	
Plots, number	11	20	16	11	7	
Total colloids, percent	7.33	6.71	7.13	8.13	6.27	None.
Total sands, do	60.9	63.6	59.1	56.7	64.7	Do.
Moisture equivalent, do	20.6	19.5	21.5	21.7	18.8	Do.
Total nitrogen, do	.158	.178	.206	.215	.220	$0.312 \pm .076$.
Standard error of mean, σm, do	±.019	±.020	±.017	±.032	±.051	
Loss-on-ignition, do	8.43	8.82	10.41	9.90	9.18	Uncertain.
Reaction, pH	4.74	4.68	4.71	4.71	4.89	None.
Exchangeable hydrogen[1], milligram-equivalents	7.41	8.84	10.14	8.67	8.95	Do.
Total base capacity[1], do	14.12	15.66	17.89	15.80	16.39	Uncertain.
Soluble nutrients per acre by quick test:						
Calcium, pounds	22.3	22.8	24.7	29.1	32.1	Slight.
Potassium, do	46.8	39.9	45.0	40.5	61.4	Uncertain.

[1] Per 100 gm. of soil.

Here it may be observed that the rather definite inverse correlation found in two cases in table 6 has disappeared, and that nitrogen shows a low but significant positive correlation. Otherwise, the results are much the same as in table 6, indicating that on the whole the soil characteristics included in this study are not of first importance in the growth of oaks on the areas in question. It must be recognized, of course, that the relative influence of soil and of environmental factors other than soil may, in some instances, be extremely difficult to evaluate. This appears to be the case in about 25 of the 65 plots. Here there was evidence that soil was not of first importance, although it lacked the certainty of the 11 plots previously mentioned. If, then, the soils of the 40 remaining plots should be compared with their site index a much closer correlation will be found, as follows:

Total nitrogen	$r=0.717\pm0.052$
Moisture equivalent	$r=\ .674\pm\ .058$
Loss on ignition	$r=\ .629\pm\ .064$
Total base capacity	$r=\ .474\pm\ .083$
Total colloids	$r=\ .095\pm\ .106$

Naturally one cannot discard plots at will in order to obtain good correlations. On the other hand, one is equally in error in blindly including all plots regardless of the environmental characteristics of each. Considering the conditions obtaining in these stands, it is evident that, other conditions being equal, the quality of the soil has a definite bearing on the site quality of these oak stands. In previous work in this region, Haig (*8*) found a fairly good correlation ($r=0.52$) between site index and the colloidal content of the A horizon in red pine plantations. On the other hand, Hicock et al. (*10*) found a very poor correlation although they worked in the same general region on the same species.

The results obtained in Finland by Valmari and treated statistically by Ilvessalo (*11*) show the following correlations between site quality and soil properties:

Loss on ignition	$r=0.453\pm0.078$
Total electrolytes	$r=\ .407\pm\ .081$
Total nitrogen	$r=\ .736\pm\ .056$
Phosphorus	None
Potassium	$r=0.214\pm0.091$
Calcium	$r=\ .612\pm\ .069$

These correlations are in the same order of magnitude as those obtained by the writer on the 40 plots; but since the latter represent only 54 percent of the plots studied, it would appear that in the Connecticut forests the soil is of less significance in determining site quality than it is in the forests of Finland.

RESPONSE OF OAK TO SOIL TREATMENT

It will be of interest at this point to discuss briefly the behavior of red oak under controlled conditions when grown in soil variously treated. In the spring of 1934 a set of 16 concrete soil frames containing Cheshire fine sandy loam which had been fertilized and cropped annually during the preceding 7 or 8 years, was planted to the southern species of red oak (*Quercus rubra* L.). The frames measured 25 by 25 inches with the walls extending about 21 inches below the surface of the soil. There was space for only 5 trees in each frame. The fertilizing materials consisted of urea, applied at the rate of 100 pounds of N per acre; phosphoric acid (H_3PO_4, 85 percent) at the rate of 200

pounds of P_2O_5 per acre; and potassium acetate ($KC_2H_3O_2$) at the rate of 100 pounds of K_2O per acre. The whips were 6 to 12 inches long when planted, and were then cut back to about 3 inches.

In the spring of 1936 all but one tree was cut off at the ground level and weighed. The results are shown in table 8.

TABLE 8.—*Effect of soil treatment on growth of red oak in soil frames at New Haven*

Treatment [1]	Green weight per tree of good trees removed in March 1936 [2]	Treatment	Green weight of 1 tree April 1937	Treatment	Mean weights of trees in all frames	
					Average per tree	Average per tree when very poor trees are omitted
	Grams		*Grams*		*Grams*	*Grams*
N	196	N	735	No lime	85.1	128
P	150	NPK	704	Lime	64.3	70
NK	150	K	581	Nitrogen, no lime	85.8	144
K	127	P	490	Nitrogen and lime	81.3	84
NP	125	NK	478	No nitrogen or lime	84.5	112
NPK	106	PK	306	Lime, no nitrogen	47.8	56
None	100	NP	230	No nitrogen	66.1	84
PK	70	None	228	Nitrogen	83.5	114
				No phosphorus	68.7	93
Mean	128		469	Phosphorus	80.9	102
				No potassium	79.3	108
LNP	115	LNP	640	Potassium	70.2	90
LNPK	110	LNPK	563			
LPK	79	LPK	547			
LN	67	LK	429			
LP	58	LNK	407			
L	55	L	362			
LNK	44	LN	337			
LK	32	LP	331			
Mean	70		452			

[1] L=lime.
[2] All but 1 tree removed March 1936. No data could be obtained on the root systems.

There is no question but that the oaks responded to the different treatments, but the actual order of response is uncertain because of the high degree of variation between individual trees in any one frame. It is believed that the acidity of the soil played an important role in this experiment. When treatments are grouped and averaged, it is seen that the mean growth in the frames receiving lime was lower than in those without lime. The superior showing of nitrogen is partly due to the greater acidity resulting from its use. Lime without nitrogen gave the lowest mean values. Phosphorus was beneficial, but potash was not. The average pH value of the limed series was 5.62; of the unlimed, 4.62.

In contrast, sugar maple (*Acer saccharum* Marshall) planted in an adjoining set of frames identical in every respect, showed a marked response to lime as indicated in table 9. Although the untreated frame was the poorest, neither phosphorus nor potassium showed any consistent beneficial effect.

In a third set of frames white ash (*Fraxinus americana* L.) appeared to be benefited by the presence of lime when all unlimed frames were compared with the limed ones. On the unlimed series all treatments receiving phosphorus, either alone or with nitrogen, were making better growth (in 1938) than those not receiving phosphorus. On the limed series nitrogen seemed to be the most necessary element.

for in all plots receiving nitrogen growth was considerably more rapid than in those without nitrogen. The most reliable data will be obtained, of course, at the conclusion of the experiment when the trees are removed and weighed.

TABLE 9.—*Effect of soil treatment on growth of sugar maple in soil frames at New Haven*

Treatment[1]	Total green weight of 3 trees without leaves or roots	Treatment	Mean weights of trees in all frames
	Grams		*Grams*
P	292	No lime	772
LNK	289	Lime	1,030
LP	254	Nitrogen, no lime	726
LN	252	Nitrogen and lime	1,133
NK	240	No nitrogen or lime	819
LK	232	Lime, no nitrogen	927
LNP	206	No phosphorus	904
LNPK	198	Phosphorus	899
PK	175	No potassium	909
L	163	Potassium	894
NP	134		
LPK	127		
N	119		
K	116		
NPK	113		
None	100		

[1] L=lime.

Because of the small number of trees in each frame, the natural variability of individuals so characteristic of hardwoods, and the brief duration of the test, these results are of limited value in ascertaining the nutrient needs of the species in question. Nevertheless, they are useful in indicating trends, and should be so evaluated.

RELATION OF SITE INDEX TO KIND OF SOIL

Thus far only the upper part of the soil profile has been considered. This is the most important part of the mineral soil so far as root feeding and seedling development are concerned and, in the present work at least, it yields the most interesting data when subjected to chemical analysis. Others, including Aaltonen (*1*), have confined their chemical analyses to samples taken from the first 10 cm. However, the subsoil plays an important role, and in many cases is the controlling influence in determining site quality. On the one hand, a heavy clay subsoil restricting drainage, aeration, and root penetration, and on the other hand, a loose, coarse sand subsoil resulting in excessive droughtiness may be equally deleterious to tree growth. Reference has already been made to the condition in the vicinity of Eberswalde where depth to the underlying marly clay controls the composition and growth of forest and ground vegetation alike. A somewhat similar condition exists at Visingsö, an island in Vättern in south Sweden (*18*). Also in south Sweden the gravelly kames and eskers have a higher base mineral index than do the sands, and this seems to be correlated directly with site quality.

Previous work (*10*) has indicated that in this region (Connecticut) several soil series may be biologically equivalent so far as tree growth is concerned. Therefore in attempting to correlate site index with

soil series, it is advisable to combine some of the series into larger groups, ignoring the finer distinctions recognized in soil mapping. In table 10 is shown the distribution of plots by site index and by soil groups. No correlation is indicated, due partly at least to the small representation of some of the soil groups. The high site index of some of the plots in the sandy group is the result of high water table, as previously mentioned.

TABLE 10.—*Relation between site index and kind of soil*

Site-index class	Number of plots of each kind of soil indicated						Total
	Sandy terrace (Merrimac, Enfield)	Upland, red sandstone soils (Cheshire, Wethersfield)	Gravelly (Hinckley, Manchester)	Flat poorly drained (Whitman, Peru)	Upland, friable subsoil (Gloucester, Hinsdale, etc.) [1]	Upland, compact subsoil (Charlton, Woodbridge, Hollis)	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
25–40					2		2
40–45					2		2
45–50	1	1			5	1	8
50–55		2	1		5	1	9
55–60	2	2	1	3	12	2	22
60–65		1	2	1	7	1	12
65–70	1	3	1		6	1	12
70–75	2	1			2		5
75–80		1			1		2
80	2						2
Total	8	11	5	4	42	6	76

[1] Other series included in this group are: Brookfield, Maltby, and Holyoke.

TABLE 11.—*Comparison of soil groups with respect to certain soil characteristics*

Soil characteristic or constituent	Horizon or depth	Group 1, friable subsoil	Group 2, compact subsoil	Group 3, red sandstone and shales	Group 4, poorly drained	Group 5, gravelly eskers, etc.	Group 6, sandy terrace
Soils in red pine plantations:							
Plots, number		7	5	4		4	3
Total N, percent	A_1	0.285	0.287	0.236		0.213	0.078
	A_3	.147	.208	.114		.148	.036
	B	.046	.044	.040		.053	.017
	C	.028	.020	.019		.019	.009
	Inches						
Citric acid soluble P, p. p. m.	0–6	52	70	32		105	37
	6–18	33	35	15		74	29
Replaceable Ca, do	0–6	125	181	135		109	36
	6–18	93	155	187		76	24
Soils from oak stands:							
Plots, number		[1] 44	6	10	4	5	8
Total N, percent	0–5	0.207	0.181	0.220	0.319	0.175	0.088
Exchangeable hydrogen, milligram equivalents [2]	0–5	9.7	8.3	8.1	17.9	9.0	4.9
Exchangeable bases, do	0–5	7.4	6.2	7.9	17.1	6.3	3.6
Total base capacity, do	0–5	17.1	14.5	16.0	34.9	15.3	8.5
Saturation, percent	0–5	42.4	43.1	49.7	48.4	42.5	41.6
Soluble Ca per acre, pound	0–5	23.0	29.0	30.0	19.0	22.0	18.0
Soluble K per acre, do	0–5	42.0	55.0	56.0	31.0	44.0	31.0

[1] Including 5 plots on the Holyoke series.
[2] Per 100 gm. of soil.

A comparison of some of the characteristics of the several groups of soil series (table 11) shows that the poorly drained soils were outstandingly more fertile than all others, although there were individual soils in groups 1 and 3 that were equally rich. In the pine-

plantation soils the compact subsoil group was richer in some cases than the other groups, but this was not uniformly true nor was it true in the oak soils. No plots were located on limestone soils and only five were on the Holyoke series. The latter is a stony soil derived from a thin glacial deposition on traprock. The traprock influence is sufficient to give these soils a higher base content than the average, but such benefit is overshadowed by soil moisture deficiencies due to the relative thinness of the soil.

RELATION OF SITE INDEX TO HUMUS TYPE

As stated under Description of the Region, the predominating humus types in Connecticut are granular mor and mull. Fibrous mor is encountered only occasionally, as is also firm mull. Within the granular mor group there are many subtypes which should be taken into consideration, but so far no adequate method of evaluation or comparison has been devised. The relation between site index and humus types is shown in table 12.

TABLE 12.—*Relation between site quality and humus type*

ALL PLOTS

Item	Mull	Fibrous mor	Granular mor	Mull-mor	Fibrous mor over podzol
Site index:					
Average	63.9	61.6	60.3	59.1	54.8
Range	55–76	47–69	48–74	30–83	36–63
Plots—number	8	9	33	15	11
AFTER ELIMINATING 4 PLOTS WITH EXTREMELY LOW SITE INDEX					
Site index:					
Average	63.9	63.0	60.3	61.1	58.3
Range	55–76	57–69	48–74	45–83	50–63
Plots—number	8	8	33	14	9
AFTER ELIMINATING 13 ABNORMAL PLOTS					
Site index:					
Average	63.9	60.0	57.5	70.0	54.8
Range	55–76	47–67	48–67	54–83	36–63
Plots—number	8	8	26	10	11

Considering not only the mean site index for the group, but also the range in site index, it is quite obvious that the mull type ranks first, and the fibrous-mor-over-podzol type last. Among the others there is little choice. By eliminating four plots that were decidedly lower than the others, the resulting means show even smaller differences, as is seen in the second part of table 12. If the 13 plots that are among the "abnormal" group are eliminated, the resulting averages are as shown in the lower part of table 12. This places mull-mor in first place, with mull second. Actually this rating is more nearly correct, for it takes into consideration some of the environmental factors other than soil that affect tree growth.

When the soils under the several humus types are compared the mean values shown in table 13 are obtained. Although individual values varied quite widely in each case, the means show rather def-

inite trends in favor of the mull and mull-mor types. It is of interest to note that of the five plots on Holyoke soil, four were classified as mull or mull-mor types.

From these studies it may be concluded, therefore, that in general good site index is associated with the better humus types.

TABLE 13.—*Relation between soil characteristics and humus type*

Soil characteristic or constituent	Mull	Mull-mor	Granular mor	Fibrous mor	Fibrous mor over podzol
Total colloids.....percent	10.2	9.0	6.5	7.0	5.4
Moisture equivalent.....do	22.1	24.3	20.7	20.0	17.8
Total nitrogen.....do	.195	.233	.193	.172	.157
Base capacity.....milligram-equivalents[1]	14.8	16.6	16.5	16.9	14.3
Calcium per acre.....pounds	54	38	17	13	13
Potassium per acre.....do	70	52	41	37	33

[1] Per 100 gm. of soil.

RELATION OF SITE INDEX TO TOPOGRAPHY

When compared on the basis of topography (table 14) a rather well-defined difference is found between the top and the foot of slopes, the foot, of course, giving the higher site index. No other relationship with topography could be found. Rocky ridges are generally considered unfavorable sites because of the thinness of the soil, the low natural moisture supply, and the greater exposure to drying winds. In this work such sites were not as poor as had been anticipated, although the range in site index was very wide. The data with respect to aspect or exposure were incomplete and therefore cannot be brought into the discussion.

TABLE 14.—*Relation between site quality and topography*

Topography	Plots	Site index	Range	Topography	Plots	Site index	Range
	Number				*Number*		
Long slope, foot	4	67.0	60–73	Rocky ridge, top	9	59.1	36–72
Level, well drained	18	63.6	47–83	Long slope, top	7	57.3	42–76
Level, poorly drained	4	60.2	58–63	Short steep slope, middle	4	51.0	30–64
Long slope, middle	21	60.0	45–74	Short steep slope, top	2	50.0	45–55
Moranic deposit, top	4	59.5	50–69	Short steep slope, foot	1	48.0	.....

RELATION OF SITE INDEX TO COMPOSITION AND RELATIVE ABUNDANCE OF LESSER VEGETATION

Although 82 species were recognized and recorded (in addition to reproduction of tree species) only a few were sufficiently prevalent to warrant an attempt at correlation with site index. In the field the occurrence of each species was estimated and reported as occasional, frequent, abundant, or very abundant. For purposes of correlation, arbitrary values were assigned to each designation and then averaged. Several closely related species or species with similar requirements were lumped together, as follows: the vacciniums and *Gaylussacia*, the several viburnums, *Rubus* species (*idaeus*, *hispidus*, and *villosus*), the mosses, and the ferns.[13] The results, shown in table 15, indicate an inverse correlation between site quality and the

[13] Separation of the ferns according to site preference proved of no advantage and therefore was not made.

vacciniums, the mosses, and to a certain extent, witch-hazel. In the case of *Rubus* species, the correlation is positive as would be expected, for these species are usually found on the better sites. The correlations are more in evidence in the first part of table 15 than they are in the second part where the plots were regrouped in order to give a more equitable distribution of plots.

TABLE 15.—*Relation between site index and presence of lesser vegetation*[1]

Site index	Plots	Relative abundance of—						
		Vaccinium, Gaylussacia	*Kalmia latifolia*	*Hamamelis virginiana*	*Viburnum*	*Rubus* spp.	Mosses	Ferns
GROUPED IN SITE-INDEX UNITS OF 10 (FIRST GROUP=20)								
	Number							
20–39	2	80	0	15	20	0	30	25
40–49	8	33	10	15	9	0	20	9
50–59	28	44	3	10	17	1	14	14
60–69	26	33	14	12	19	3	10	19
70–79	7	11	1	8	7	6	13	17
80+	2	0	0	0	0	5	5	0
GROUPED IN SITE-INDEX UNITS OF 5 (EXCEPT FIRST AND LAST GROUPS) IN ORDER TO OBTAIN MORE EQUITABLE DISTRIBUTION								
20–49	11	42	8	14	0	0	20	11
50–54	9	44	7	0	28	0	13	20
55–59	18	45	1	16	13	1	15	12
60–64	16	39	13	10	14	4	11	18
65–69	8	30	10	11	25	0	5	18
70+	12	12	8	10	10	7	12	15

[1] Arbitrary values used for purposes of averaging: Very abundant, 80; abundant, 50; frequent, 30; occasional, 10.

RELATION OF KIND OF SOIL TO OCCURRENCE OF LESSER VEGETATION

Using the soil groupings as given in table 10, the writer has listed the "relative occurrence" of the reproduction of tree species in Table 16 and that of the lesser vegetation in table 17. By relative occurrence is meant the relative abundance and frequency of a species in relation to the number of plots in any particular soil group. It was determined as follows: As shown in table 15, arbitrary values were assigned to each estimate of abundance; viz, Very abundant=80, abundant=50, frequent=30, and occasional=10. The sum of these values for each species on each soil group was divided by the number of plots of that particular soil group, giving the relative occurrence. For example,
Amelanchier spp. on gravelly soil:

Abundance values—10, 10, 30, 10. Sum=60

Number of plots on gravelly soil=5. 60÷5=12 relative occurrence. By this calculation it was possible to compare soil groups with each other even though no two groups had the same number of plots.

Although the relatively small number of plots in most of the soil groups restricts the reliability of the results, these are nevertheless, of considerable interest. According to the data in table 16, gray birch, red maple, and especially oak reproduction was quite abundant on the sandy soils. Black cherry and sassafras were found in greater abundance on the heavy subsoils than any of the other species. No

reproduction was found on the poorly drained soils. All species reproduced on the loose subsoil type represented by the Gloucester and closely related soil series.

TABLE 16.—*Relative occurrence* [1] *of reproduction of tree species on different types of soil*

Species	Relative occurrence—				
	Compact subsoil	Red sandstone soils	Loose subsoil	Gravelly soil	Sandy soil
Ash	1.6		2.1		
Birch, black			1.6		
Birch, gray			2.1		7.5
Cherry, black	6.7	6.4	9.5	6	
Hemlock		2.7	.5	2	
Maple, red	3.3	3.6	13.8	10	8.8
Maple, sugar	3.3		3.1		
Oak	1.2	11.8	20.7	16	21.2
Pine, pitch			.5		
Pine, white			1.4	4	1.3
Sassafras	5.0	2.7	6.4	2	1.3

[1] Relative occurrence = $\frac{\text{sum of occurrence}}{\text{Number of plots in the soil group}}$. No reproduction on poorly drained soil.

TABLE 17.—*Relative occurrence* [1] *of lesser vegetation on different types of soil*

[Number of plots in parentheses]

Species	Relative occurrence on—					
	Poorly drained	Compact subsoil	Red sandstone soils	Loose subsoil	Gravelly soil	Sandy soil
Amelanchier spp.		1.6 (1)	1.0 (1)	6.2 (10)	12 (4)	6.3 (3)
Amphicarpa monoica				1.9 (4)	6 (1)	3.8 (1)
Aralia spp.				2.6 (7)	6 (1)	2.5 (2)
Aster spp.			7.3 (4)	3.3 (6)		
Benzoin aestivale	10 (1)			.7 (1)		
Carpinus caroliniana	10 (1)	1.6 (1)		.3 (1)		
Chimaphila maculata		1.6 (1)		1.9 (4)		6.3 (3)
Chimaphila umbellata		1.6 (1)		5.0 (9)	4 (2)	3.8 (1)
Clethra alnifolia	20 (2)			.7 (1)		
Cornus alternifolia and *paniculata*		1.6 (1)	3.6 (2)	.3 (1)	2 (1)	
Cornus florida		10.0 (2)	10.9 (4)	12.1 (15)	8 (2)	
Corylus americana			3.6 (2)	3.3 (6)	2 (1)	10.0 (4)
Crataegus spp.		3.2 (2)		1.4 (2)		
Ferns	30 (3)		25.4 (12)	26.2 (32)	8 (2)	5.0 (2)
Gaultheria procumbens			1.0 (1)	5.2 (8)	6 (1)	7.5 (2)
Gramineae		16.6 (4)	20.9 (9)	24.2 (35)	30 (5)	18.3 (7)
Hamamelis virginiana		5.0 (1)	8.2 (3)	13.6 (17)		
Kalmia angustifolia			2.7 (1)	1.9 (4)		5.0 (2)
Kalmia latifolia			5.4 (2)	10.5 (14)	2 (1)	1.2 (1)
Lycopodium				.5 (2)	2 (1)	1.2 (1)
Maianthemum			2.7 (1)	.7 (3)		
Mitchella repens		3.2 (2)	3.6 (2)	2.4 (6)	2 (1)	
Mosses		1.6 (1)	7.3 (4)	17.4 (29)	10 (3)	11.2 (5)
Myrica asplenifolia				.5 (2)		1.2 (1)
Ostrya virginiana		1.6 (1)	4.5 (1)	4.8 (8)	4 (2)	
Pyrola spp.		1.6 (1)		1.0 (2)		2.5 (2)
Pyrus arbutifolia			1.0 (1)	1.4 (2)		
Rhododendron canescens		5.0 (1)	5.5 (2)	6.4 (11)		
Rosa spp.		1.6 (1)				1.2 (1)
Rubus spp.		3.3 (2)		2.9 (6)		3.8 (3)
Smilacina spp.				2.9 (4)		
Smilax spp.				2.9 (4)	2 (1)	
Vaccinium spp. and *Gaylussacia* spp.	20 (2)	40.0 (7)	34.5 (14)	72.4 (60)	60 (8)	37.5 (6)
Viburnum acerifolium		3.3 (2)	17.3 (7)	19.0 (26)	4 (2)	7.5 (2)
Viburnum cassinoides, dentatum and *lentago*	20 (2)		4.5 (3)	.3 (1)		

[1] Relative occurrence = $\frac{\text{Sum of occurrence}}{\text{Number of plots in the soil group}}$

When it comes to the lesser vegetation exclusive of tree seedlings, (table 17), six species or species groups occurred on poorly drained soil. *Vaccinium* and/or *Gaylussacia* were found on all soil types but tended to be in greater abundance on the drier soils. Ferns were found on all except the compact subsoil group. Grasses show a quite uniform distribution on all except wet soils. The failure to find on either gravelly or sandy soils *Aster, Benzoin, Carpinus, Clethra, Crataegus, Hamamelis, Maianthemum, Pyrus, Rhododendron, Smilacina,* and all species of *Viburnum* except *acerifolium* may or may not be significant. Practically all of these species are known, however, to prefer moist situations.

DISCUSSION

From the literature we learn that, by and large, the chief limiting soil factors in forests at moderate elevations in the Temperate Zone are soil moisture, lime content, and porosity or aeration of the soil. In some sections one factor predominates, in other sections another. In Connecticut with its light soils, porosity or soil aeration is not a factor (except in strictly local situations). Lime and other bases are of importance for the more exacting hardwood species; but lime alone has relatively little effect on the conifers and for the most part on the oaks. This was demonstrated for oak in the soil frame experiment previously described. In other words, a low lime content cannot be said to be a limiting factor in oak stands in Connecticut.

In certain situations where an undesirable humus condition is caused by or maintained through the presence of *Vaccinium* and *Gaylussacia*, a higher base content in the soil would probably favor the presence of more desirable shrub and ground-cover species and thus benefit the oaks indirectly. Furthermore, *Vaccinium* and *Gaylussacia* offer severe competition to the oaks, and their elimination would be beneficial. The difficulty in establishing pine on the heath lands of northwestern Germany and Denmark is well known. Wiedermann (*22*) states that berry bushes (blueberry and huckleberry) growing under a good tree species frequently exert an unfavorable influence on the soil; whereas a raspberry or grass flora may bring about favorable conditions even under a poor species.

Stålfelt (*17*) reports that the loss of water from a soil with a shrub cover is much greater than from a bare soil or from one covered only with forest litter or with moss. Wittich (*23*) states that on the sandy soils of north Germany, bare soil has a lower water content up to about June 1 than soil supporting shrub growth; but from then on into autumn the bare soil is the more moist.

Although this study did not include measurements on soil moisture, it was obvious in many situations that moisture is a very important factor. While the ideal plan for such studies would require frequent measurements throughout the year, such a program is not always feasible. The determination of the relative moisture content during the driest and hottest part of the summer is a good substitute, and such determinations were made in 1934 and 1935 in a number of forests, but without reference to the particular oak stands discussed in this paper and without growth measurements. The field moisture content was compared with the moisture equivalent to give the relative wetness $\frac{\text{field moisture}}{\text{moisture equivalent}} \times 100 = R.W.$ Some of the results are given in table 18.

TABLE 18.—*Relation between apparent site quality, and relative wetness* [1] *of some forest soils during the driest part of the summer*

[Number of samples [2] in parentheses]

Location and kind of forest	Apparent site quality	1934, 0–5 inches	1935	
			0–5 inches	12–15 inches
Meshomasic Forest, Cox area, mixed hardwoods:				
Upper slopes and ridges	Fair to poor	96(1)	77(1)	66
Middle slopes (gentle)	Medium	91(1)	123(3)	122
Lower slopes	Good	166(3)	166(3)	139
Meshomasic Forest, mountain top, scarlet oak	Poor	107		
Meshomasic Forest, upper slope, mixed hardwoods [3]	Medium	117		
Mount Carmel, hilltop, mixed hardwoods	Fair	66(1)	55(1)	55
Mount Carmel, lower slope, mixed hardwoods	Good	82(1)	61(1)	47
Rainbow plantation, Merrimac coarse sand:				
Red pine check plot	Poor	58	47	46
Red pine raked plot [4]	do	52	41	44
Red pine triple-thickness plot [5]	do	58	54	47
Oak plot [6]	do	49	65	67

[1] Relative wetness $= \frac{\text{field moisture content}}{\text{moisture equivalent}} \times 100$.

[2] Each sample represents from 2 to 4 borings.

[3] Red oak, scarlet oak, white oak, red maple.

[4] Soil raked clear of all organic debris every fall.

[5] Litter added yearly from 2 raked plots, making the A_0 horizon 3 times its normal thickness.

[6] Rather open stand of red oak, scarlet oak, and white oak, all 6–14-inch d. b. h., underplanted with white pine 2–3-feet tall.

Owing to the inequalities in precipitation, especially in summer, it is not safe to compare one forest or locality with another without having a rain gage in each. But within any one locality comparisons are possible, and they are valuable in indicating the soil moisture conditions during the most critical period. These data show that a more or less direct correlation exists between the apparent site quality and the relative wetness of the soil, and they lend support to the belief that moisture is undoubtedly the chief limiting soil factor in tree growth in Connecticut in spite of the relatively abundant rainfall that is quite uniformly distributed throughout the year. It is difficult, of course, to separate moisture per se from its accompanying effects: viz, favorable humus condition, good microbiological and macrobiological activity, and availability of soil nutrients.

Excess moisture, on the other hand, results in limitation of root development, reduction of ferric iron, nitrates, and other compounds, and cessation of biological activity. Morgan [14] when investigating the cause of a number of deaths of young trees in an oak plantation in New Jersey, found a very high content of ferrous iron in the soil surrounding the roots of the dead trees. He attributed this to the fact that a considerable quantity of organic matter in the form of sod had been incorporated in the soil at the time of planting, and the wet soil with resulting poor aeration caused a reduction of some of the iron to the ferrous condition. In the case of the living trees, little or no sod got into the planting hole.

Soil tests reveal that the average forest soil in Connecticut is more acid and is considerably lower in fertility than the average farm soil as shown in table 19.

With respect to soil and humus types, it is of interest to note that scarlet oak was the predominant species on 9 out of 11 plots showing evidences of podzolization. Nine of the eleven were on the Glouces-

[14] MORGAN, M. F. Private communication.

ter-Hinsdale group of soils, 1 on gravelly soil (Hinckley) and 1 on wind-blown soil (Enfield). On 10 of these plots huckleberry and lowbush blueberry, especially the former, were by far the most abundant element in the ground cover. Accompanying shrubs were mostly ericads (*Kalmia latifolia, K. angustifolia, Vaccinium corymbosum*, etc.). Bracken fern was generally common also.

TABLE 19.—*Comparison of soluble nutrients in typical forest, farm, and greenhouse soils by Morgan's rapid soil test* [1]

Soluble nutrient	Forest soil, pH 4.5	Farm soil, pH 5.6	Greenhouse soil, pH 6.5	Soluble nutrient	Forest soil, pH 4.5	Farm soil, pH 5.6	Greenhouse soil, pH 6.5
	Pounds per acre	*Pounds per acre*	*Pounds per acre*		*Pounds per acre*	*Pounds per acre*	*Pounds per acre*
Nitric nitrogen	1	25	90	Potassium	75	200	500
Ammonia nitrogen	20	15	50	Calcium	500	3,000	4,500
Phosphorus	25	100	250	Aluminum	200	50	50

[1] The reader is cautioned not to compare the above results with those given for rapid soil tests in table 6. As previously explained, changes in procedure and color scales about 1934 so altered the readings obtained that direct comparison is not possible.

Only one pure scarlet oak stand was associated with a mull condition, and that was a young plantation on a moist and somewhat heavier agricultural soil (Wethersfield). No stand of predominantly red oak was associated with anything poorer than granular mor. The ground cover found on mull and mull-mor was typically *Viburnum acerifolium, Cornus florida*, and a great variety of smaller shrubs, wild flowers, and herbs; but *Gaylussacia* and *Vaccinium* spp. are generally present and often abundant. *Viburnum, Cornus*, shrubs, wild flowers, and herbs were conspicuously absent on the fibrous-mor-over-podzol plots where the ericads predominated.

SUMMARY

Seventy-six temporary plots in even-aged second-growth oak stands well distributed throughout the State of Connecticut furnish the basis for this study. Height, diameter at breast height, and age of the dominant and codominant oaks were ascertained separately for each species. The five principal species were white, red, black, scarlet, and chestnut oak, and there were a few swamp white and pin oak. Notes were taken on lesser vegetation, topography, and soil; and the soil was sampled to a depth of about 5 inches.

Site indices were calculated from height curves and formulas supplied by the Forest Service of the United States Department of Agriculture. In the absence of proof to the contrary, it was assumed that the Forest Service curves were applicable to the Connecticut data.

The climate of Connecticut is sufficiently warm and humid to favor tree growth. The soils are prevailingly acidic, largely fine sandy loam in texture, and relatively low in fertility.

The results of this study may be summarized as follows:

Site indices ranged from 30 to 83 and averaged 59.7. Nearly three-quarters of the plots had a site index between 50 and 70, and slightly over three-quarters were between the ages of 20 and 60 years. Black oak averaged highest in site index, and chestnut oak lowest. The average height and diameter at breast height of all trees was 53.2 feet and 7.98 inches, respectively.

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When all plots were included, correlations between site index and characteristics of the surface soil were, for the most part, either entirely absent or slightly inverse. In some cases it was obvious that other environmental factors, such as high water table or seepage from higher ground, were of greater consequence than the soil itself in controlling tree growth. The omission of 11 plots that exhibited obvious external influences resulted in the elimination of the inverse correlations mentioned above, and at the same time revealed a low but significant positive correlation between site index and total nitrogen. This indicated that, taking the stands as they were, the soil was not of first importance in the growth of oaks. On the other hand, where conditions are otherwise similar, the quality of the upper A horizon of the soil is more or less directly correlated with the site quality of these oak stands.

Soil series groups showed no correlation with site index.

With respect to humus types, plots having a mull condition averaged the highest site index, and the fibrous-mor-over-podzol type, the lowest.

Topographically, there was little correlation other than the obvious one, that plots at the foot of a slope have an average site index considerably greater than those on hilltops. Level, well-drained areas were slightly superior to level, poorly drained areas.

Site index was inversely correlated with the presence of *Vaccinium* and *Gaylussacia*, the mosses, and, to a certain extent, witch-hazel; but there was evidence of a direct correlation with the presence of *Rubus*.

No reproduction of tree species was observed on the poorly drained soils. On sandy soils, gray birch, red maple, and oak were fairly abundant; on the compact subsoil group black cherry and sassafras were in greater abundance than most of the other species.

Shrubs and herbaceous plants were found pretty well in accordance with their preferred environment, based largely on water relations.

With respect to vegetation and soil condition, the fibrous-mor-over-podzol type was characterized by a predominance of scarlet oak in the stand, and huckleberry and blueberry in the ground cover. On the mull type no one species of oak predominated; but the ground cover was typically *Viburnum acerifolium*, *Cornus florida*, and numerous smaller shrubs, wild flowers, and herbs, and in addition some blueberry and huckleberry.

CONCLUSIONS

In the second-growth oak stands of Connecticut site quality is the resultant of a number of environmental factors, chief of which are those affecting the water economy of the tree; viz, available moisture, topography, and depth of soil. It is apparent that the native fertility of the soil is an important determining factor in *stand composition* (as evidenced by the fact that only the less exacting species can survive on the poorer soils); but the fertility of the soil as measured by the tests used in this study is a controlling factor in *site quality* only in those situations where other environmental influences are of minor significance. Stated another way, when other conditions are equal, soil fertility is a controlling influence in determining the site quality of oak stands in Connecticut.

Topography comes into the picture only as it influences soil-moisture supply and exposure to or protection from wind and sun.

Except for a few species, the ground cover in these oak stands appears to be of little value in indicating site quality.

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HOST RANGE STUDIES WITH BACTERIUM SOLANACEARUM [1]

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INTRODUCTION

Bacterium solanacearum E. F. Smith [3] is the cause of a wilt disease of tobacco (*Nicotiana tabacum* L.), potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), redpepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), banana (*Musa sapientum* L.), peanut (*Arachis hypogaea* L.), and a number of ornamental and wild plants. The disease on tobacco is known in the United States as Granville wilt, which is severe in certain areas of North Carolina and adjoining States. Crop rotation is the major control measure for the disease. Inasmuch as both wild and cultivated plants are known to be susceptible, the host range of the causal organism is an important factor in its control. The principal object of this investigation was to determine the susceptibility of the crops and field weeds of the wilt-infested area to natural infection. This information is pertinent to the crop-rotation experiments in progress at the present time.

Successful stem inoculation has been accepted as proof of susceptibility to the disease. However, several species on which stem inoculation has been successfully carried out were not known to become diseased when grown on infested soil, even though certain of these species are widely grown as crop plants. It appeared that certain species might be susceptible to artificial stem inoculation and yet not become naturally diseased by infection through the roots from the soil-borne parasite. This possibility made it necessary to compare the susceptibility of plants to natural and artificial infection. The experiments were in progress during 1935, 1936, 1937, and 1938.

The literature on *Bacterium solanacearum* has been reviewed by Palm and Jochems (*13*),[4] Elliot (*4*), and Labrousse (*12*). The reader is referred to these publications for world-wide host lists and bibliographies. Host range studies applicable specifically to Granville wilt have been conducted by Fulton and Winston (*6*), Fulton and Stanford (*5*), Stanford and Wolf (*20*), and Wolf (*22*).

METHODS

All species under test were grown in rows for one or more seasons on soil known to be very highly infested with *Bacterium solanacearum*.

[1] Received for publication January 24, 1939. This paper is a revision of a dissertation submitted to the University of North Carolina in partial fulfillment of the requirements for the degree of doctor of philosophy in botany. The experimental work was conducted at the Tobacco Branch Station, Oxford, N. C., and the North Carolina State College of Agriculture and Engineering, Raleigh, N. C.

[2] The writer is indebted to Dr. R. F. Poole, of the North Carolina State College, E. G. Moss, of the Tobacco Branch Station, and Dr. E. E. Clayton, of the Division of Tobacco and Plant Nutrition, for suggestions and criticisms, and also to Dr. M. F. Buell, of the college, and R. K. Godfrey, formerly of the Division of Tobacco and Plant Nutrition, for aid in the identification of the plants.

[3] *Phytomonas solanacearum* (E. F. Smith) Bergey et al.

[4] Italic numbers in parentheses refer to Literature Cited, p. 439.

Cultivation with horse-drawn and hand implements encouraged natural infection after root injury. In order to measure the severity of wilt during each season, every third or fifth row of the host range plantings was set with varieties of tobacco known to be highly susceptible. Figure 1 is a field scene illustrating the severe disease conditions under which the tests were conducted. In taking final notes on susceptibility to natural infection, counts were made of plants showing symptoms on the below-ground as well as on the above-ground parts. The results of the natural-infection tests have been

FIGURE 1.—Field scene illustrating arrangement of tobacco checks and severity of wilt on naturally infested field used for the experiments. *a*, Tobacco, 95-percent diseased; *b*, red pepper, 50-percent diseased; *c*, tomato, 100-percent diseased; *d*, tobacco, 92-percent diseased. Photographed August 7, 1935.

supplemented by hundreds of observations made on plants growing on wilt-infested farms.

Tests of susceptibility to inoculation were usually made on 5 to 10 individuals in the plantings described in the previous paragraph. Inoculations were made by inserting small wedges of discolored tissue from the woody cylinder of diseased tobacco plants into the stems of the plants just below the terminal buds. Where possible, inoculations were made on small plants during early summer, a period during which wilt is quite active in the field. Infected tissue used immediately after its removal from the plants provided a constant source of virulent inoculum. Pure cultures were not satisfactory because *Bacterium solanacearum* varies in pathogenicity when maintained in artificial culture.

Bacterium solanacearum was proved from all infected species. Tissue inoculations were made from the diseased plants into tobacco

growing on disease-free soil for all known host plants showing typical wilt symptoms. Tobacco for these confirmatory inoculations was grown on Cecil or Iredell sandy loam soils not previously cropped to tobacco and on which the disease is not generally present. More specific proof of *Bact. solanacearum* was required for all new or questionable hosts. Pure-culture isolation and needle-prick inoculations were made into tobacco for these species. In addition, laboratory tests for gas production on glucose broth, nitrate reduction, gelatin

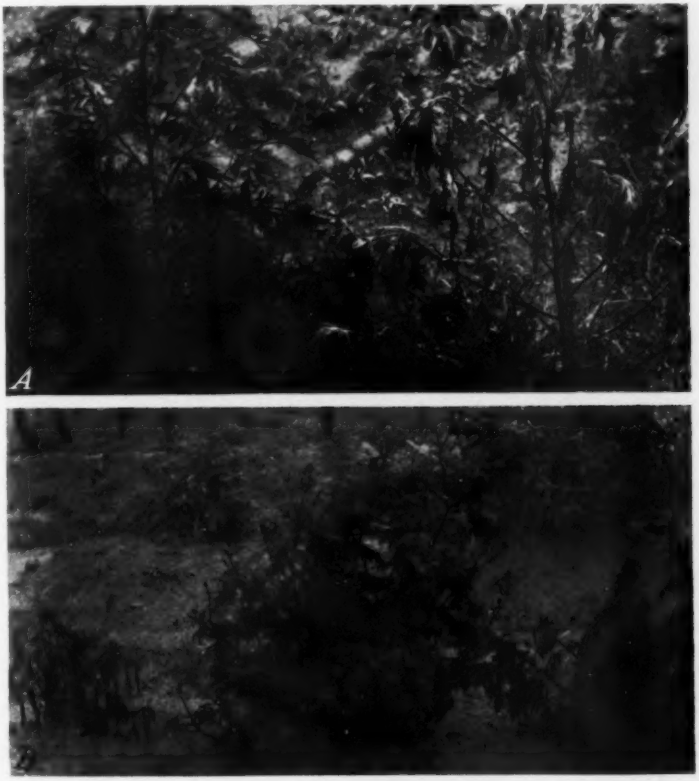

FIGURE 2.—Granville wilt on weeds from natural infection. *A*, Common ragweed (*Ambrosia elatior* L.), a common field weed of slight susceptibility; healthy plant on left, diseased plant on right. *B*, Spanish-needles (*Bidens bipinnata* L.), which became 100-percent diseased in all plantings; healthy plant in center, diseased plants on left and right.

liquefaction, and pigment production on steamed potato cylinders were carried out. The last-named test was especially useful in distinguishing *Bact. solanacearum* in the laboratory. A characteristic brownish-black pigment is produced within a few days after inoculation on this medium.

RESULTS

In discussing the susceptibility of the different species to the wilt disease it is convenient to divide them into three classes: Class 1, species that are highly and definitely susceptible, and hence host plants; class 2, the species about which the results raise a question as to whether they should be regarded as hosts; and class 3, species that are definitely immune.

SUSCEPTIBLE SPECIES

The species reacting positively to both natural-infection and stem-inoculation tests were considered susceptible. These are listed in table 1, together with the type of infection observed by the original investigator for each host, and reference to the first literature citation. For convenience four susceptibility classes are given. These are defined in the table.

TABLE 1.—*Species susceptible to natural and artificial infection by Bacterium solanacearum*

Botanical name	Common name	Type of infection observed by original investigator [1]	Approximate number of plants grown	Susceptibility to natural infection
Nicotiana tabacum L.	Tobacco	I (*10*) N (*17*)	1,000	High (71 to 100 percent diseased).
Lycopersicon esculentum Mill.	Tomato	N (*16*)	51	Do.
Nicotiana rustica L.	Aztec tobacco	N (*9*)	89	Do.
Solanum melongena L.	Eggplant	N (*16*)	102	Do.
Datura stramonium L.	Jimsonweed	I (*16*)	147	Do.
Solanum nigrum L.	Black nightshade	I (*16*)	129	Do.
Croton glandulosus L.	Croton	I (*20*)	24	Do.
Bidens bipinnata L.	Spanish-needles	I (*20*)	49	Do.
Xanthium pennsylvanicum Wallr.	Cocklebur		9	Do.
Tropaeolum minus L.	Nasturtium	N (*1*)	58	Do.
Solanum tuberosum L.	Potato	N (*16*)	84	Do.
Eclipta alba (L.) Hassk.		N (*5*)	11	Do.
Capsicum annuum L.	Red pepper	N (*8*)	85	Moderate (51 to 70 percent diseased).
Physalis pruinosa L.	Ground cherry		20	Do.
Helianthus annuus L.	Sunflower	I (*18*)	87	Do.
Phaseolus vulgaris L.	String bean	N (*19*)	153	Slight (21 to 50 percent diseased).
Ambrosia elatior L.	Common ragweed	N (*5*)	148	Do.
Aster pilosus Willd.	Aster		13	Do.
Dahlia rosea Cav.	Dahlia	N (*22*)	77	Do.
Ricinus communis L.	Castor-bean	N (*18*)	117	Do.
Tagetes erecta L.	Marigold	N (*14*)	73	Do.
Petunia hybrida Vilm.	Petunia	N (*16*)	17	Do.
Arachis hypogaea L.	Peanut	N (*18*)	121	Very slight (1 to 20 percent diseased).
Leptilon canadense (L.) Britton	Horseweed	I (*20*)	129	Do.
Ambrosia trifida L.	Giant ragweed		40	Do.
Solanum carolinense L.	Horsenettle	I (*20*)	89	Do.
Xanthium chinense Mill.	Cocklebur		9	Do.
Verbena hybrida Hort.	Verbena	I (*1*)	36	Do.
Cosmos bipinnatus Cav.	Cosmos	N (*22*)	94	Do.

[1] I=reported susceptible in the literature on the basis of stem inoculation only; N=reported susceptible in the literature on the basis of observed natural infection.

Twenty-nine species were susceptible. Of these, tobacco, tomato, *Nicotiana rustica*, eggplant, Spanish-needles (fig. 2, *B*), jimsonweed, black nightshade, croton, one species of cocklebur (*Xanthium pennsylvanicum* Wallr.) (fig. 3), and nasturtium are very highly susceptible, becoming 100 percent diseased in all plantings. Tomato,

black nightshade, and Spanish-needles were the most susceptible, usually becoming 100 percent diseased 2 weeks earlier than adjacent plantings of tobacco.

Redpepper (fig. 1) and other species were much more tolerant. Root rot rather than the foliar wilting was usually the predominant symptom on the less susceptible species. This was especially true of castor-bean, horseweed, ragweed, and horsenettle.

Wide variation in disease percentages for cocklebur during different seasons and from field to field during the same year was at first quite puzzling. Later it was discovered that at least two species were present in the area. These were planted on highly infested soil in 1938. *Xanthium pennsylvanicum* was very highly susceptible and *X. chinense* was only slightly susceptible (fig. 3). This variation is of interest because the highly susceptible species is not of widespread occurrence in the wilt-infested area, while the less susceptible species

FIGURE 3.—Variation in susceptibility of *Xanthium* species to Granville wilt by natural infection: *a*, *Xanthium pennsylvanicum* Wallr., very highly susceptible; *b*, *X. chinense* Mill., slightly susceptible.

is generally present. Five species of native weeds are reported here for the first time as new hosts. These are *Xanthium pennsylvanicum*, *X. chinense*, *Physalis pruinosa*, *Aster pilosus*, and *Ambrosia trifida*.

SPECIES SUSCEPTIBLE TO ARTIFICIAL STEM INOCULATION AND APPARENTLY IMMUNE TO NATURAL INFECTION

Each species listed in table 2 was consistently susceptible to artificial stem inoculation throughout these experiments. On the other hand, they apparently never became naturally diseased by infection through the roots from the soil-borne parasite. The fact that cowpeas, soybeans, and velvetbeans were susceptible to inoculation is especially interesting, since they are important summer legumes and are often grown on tobacco land. Detailed studies were restricted to these latter species and consisted of extensive stem-inoculation and natural-infection tests on several varieties of each species and measurements

of the population of *Bacterium solanacearum* established in these plants following stem inoculation.

TABLE 2.—*Species susceptible to artificial infection and immune to natural infection by Bacterium solanacearum*

Botanical name	Common name	Type of infection observed by original investigator [1]	Approximate number of plants grown	Susceptibility to natural infection
Vigna sinensis Endl.	Cowpea	I (*19*)	770	Immune (none diseased).
Soja max (L.) Piper	Soybean	N (*15*) I (*19*) N (*23*)	760	Do.
Stizolobium deeringianum Bort.	Velvetbean	I (*20*)	605	Do.
Phaseolus lunatus L.	Lima bean	I (*19*)	175	Do.
Canna sp.	Canna		9	Do.

[1] I=reported susceptible in the literature on basis of stem inoculation only; N=reported susceptible in the literature on the basis of observed natural infection.

Field and greenhouse plantings were made of various varieties[5] of cowpeas, soybeans, and velvetbeans. All plantings remained free of natural infection as far as could be detected on both above- and below-ground parts (fig. 4). Inoculations made in the greenhouse

[5] Cowpeas: Victor, Brabham, Black, Iron, Whippoorwill, and New Era. Soybeans: Otootan, Mammoth Yellow, Laredo, Tokyo, Biloxi, and Virginia. Velvetbeans: Osceola, Hundred-Day Speckled, Bush, Early Arlington, and Tracy Black.

FIGURE 4.—Field plantings of summer legumes with checkrows of tobacco: *a*, Tobacco; *b*, cowpeas; *c*, soybeans; *d*, tobacco; *e*, velvetbeans. The legumes were susceptible to inoculation but remained free of wilt from natural infection when grown on soil where the adjacent checks of tobacco were, respectively, 95- and 96-percent diseased on August 1, 1935.

with pure cultures of *Bacterium solanacearum* showed that seedlings of all varieties of these three species were susceptible and that the velvet-bean varieties as a group were the most susceptible. However, seedlings of all species were killed by the disease. The plants grown in the field were hardier and hence less susceptible to inoculation. Under field conditions the symptoms were always localized. They consisted of wilting and bacterial occupation of the xylem vessels adjacent to the point of inoculation for all varieties except the Biloxi soybean. In both greenhouse and field inoculations (fig. 5, *A*) plants

FIGURE 5.—Inoculation and natural-infection tests with the Biloxi soybean. Plants of this variety were killed by the disease or developed localized symptoms following stem inoculation (*A*) but were immune when grown, (*B*, *b*) on soil so highly infested with *Bacterium solanacearum* that tobacco (*B*, *a*) was 100-percent dead by August 1, 1937.

of this variety were killed by the disease. However, no evidence of natural infection was found when the Biloxi variety was grown during 2 separate years on soil so highly infested with *Bact. solanacearum* that the adjacent tobacco checkrow was 100 percent dead by August 1 of each year (fig. 5, *B*).

These results confirm the work of Smith and McCulloch (*19*) and Stanford and Wolf (*20*) with cowpeas, soybeans, and velvetbeans for the stem-inoculation tests. However, the reports of natural infection on cowpeas by Schwarz (*15*) and on soybeans by Wolf (*22*) were not confirmed. No inoculations were reported by either author. It is doubtful whether the identity of the organism could be definitely established by its appearance in culture alone. Also, since there are various wilt diseases of cowpeas and soybeans caused by micro-organisms and insects, the occurrence of wilted plants is not necessarily an indicator of *Bacterium solanacearum*.

POPULATIONS OF "BACTERIUM SOLANACEARUM" IN THE LEGUMES FOLLOWING STEM INOCULATION

Since cowpeas, soybeans, and velvetbeans were immune to natural infection, it was of interest to determine the size of the bacterial populations established in these plants following stem inoculations made the latter part of June in 1936 and 1937. For this determination, dilution plates were poured from samples of infested areas in the stem. Uniform amounts of tissue were taken 1 inch below the point of inoculation because the organism spreads more rapidly down than up the stem. The 1936 samples were taken 2 weeks after inoculation and the 1937 samples 5 weeks after inoculation. In each series an isolation from naturally infected tobacco was made for comparison. The bacterial population of diseased tobacco tissues should represent the population level reached by *Bacterium solanacearum* in a susceptible plant. All isolations were made from field material.

The data for the counts, which are summarized in table 3, show that after stem inoculation a very small population of *Bacterium solanacearum* in proportion to that of naturally infected tobacco became established in the resistant plants. The wide difference between the bacterial populations of tobacco and the legumes indicates that the latter are not well adapted as food plants for this organism. Nevertheless it is able to persist for at least 5 weeks, but with the exception of the Biloxi variety of soybean (fig. 5) the population apparently never became large enough to cause widespread pathological symptoms.

TABLE 3.—*Populations of Bacterium solanacearum in inoculated stems of cowpeas, soybeans, and velvetbeans*

Plant species	Isolations made		Bacteria at uniform dilution	
	1936	1937	1936	1937
	Number	*Number*	*Number* [1]	*Number* [1]
Tobacco	1	1	874,000	190,000,000
Cowpea	5	3	30,000	711,666
Soybean	2	3	12,000	761,666
Velvetbean	3	3	35,000	10,666

[1] Average.

IMMUNE SPECIES

The species reacting negatively to both inoculation and natural-infection tests were considered immune to *Bacterium solanacearum*. These are listed in table 4. Five of the species found to be immune have been listed by various workers as susceptible. These are watermelon, *Crotalaria striata*, cotton, sweetpotato, and fireweed. References to literature citations in which reports of susceptibility are made are given in table 4. After receiving heavy stem inoculations

TABLE 4.—*Plant species immune to natural and artificial infection by Bacterium solanacearum*

Botanical name and report of susceptibility [1]	Common name	Approximate number of plants grown
Cultivated plants:		
Citrullus vulgaris Schrad. (*25*)	Watermelon	22
Crotalaria intermedia Kotschy		150
Crotalaria incana L.		150
Crotalaria lanceolata E. Mey		150
Crotalaria mazallaris Klotzsch		150
Crotalaria rutusa L.		150
Crotalaria spectabilis Roth		150
Crotalaria striata DC. (*3*)		150
Crotalaria usaramaensis E. G. Baker		100
Cucumis melo L.	Cantaloup	85
Gossypium hirsutum L. (*18*)	Cotton	84
Hibiscus esculentus L.	Okra	81
Humulus lupulus L.	Hops	12
Ipomoea batatas (L.) Lam. (*13*)	Sweetpotato	71
Lespedeza striata Hook. and Arn	Japan clover	11
Pisum sativum var. *arvense* Poir	Canadian field peas	[2] 5
Sorghum vulgare var. *saccharatum* (L.) Boerl	Sorgo	58
Trifolium incarnatum L.	Crimson clover	[2] 5
Vicia sativa L.	Vetch	[2] 5
Zea mays L.	Corn	71
Wild plants:		
Amaranthus spinosus L.	Spiny pigweed	43
Amaranthus retroflexus L.	Pigweed	25
Apocynum sp.	Dogbane	4
Bidens frondosa L.	Sticktight	14
Chenopodium album L.	Lambsquarters	102
Chenopodium botrys L.	Jerusalem-oak	30
Chrysanthemum leucanthemum L.	Oxeye daisy	8
Desmodium dillenii Darl.	Beggarticks	6
Desmodium ciliare DC.	do	8
Diodia virginiana L.	Larger buttonweed	8
Eupatorium capillifolium (Lam.) Small	Dogfennel	8
Erechtites hieracifolia (L.) Raf. (*15*)	Fireweed	108
Euthamia minor (Michx.) Greene	Flat-topped goldenrod	11
Ipomoea hederacea Jacq.	Morning-glory	12
Ipomoea pandurata (L.) Meyer	Wild potato	4
Ipomoea purpurea (L.) Lam.	Morning-glory	12
Lonicera japonica Thunb.	Japanese honeysuckle	6
Lactuca sagittifolia Ell.	Arrow-leaved lettuce	4
Oenothera biennis L.	Common oenothera	2
Oxalis stricta L.	Woodsorrel	10
Passiflora incarnata L.	Maypop	3
Phytolacca americana L.	Pokeweed	26
Polygonum pennsylvanicum L.	Smartweed	12
Polygonum persicaria L.	do	36
Portulaca oleracea L.	Purslane	6
Rubus sp.	Blackberry	4
Rumex acetosella L.	Sheep sorrel	6
Rumex obtusifolius L.		4
Sida spinosa L.	Ironweed	23
Solidago canadensis L.	Goldenrod	9
Solidago nemoralis Ait.	do	4
Solidago pinetorum Small	do	4
Tecoma radicans (L.) Juss.	Cow itch vine	8
Trifolium arvense L.	Rabbit-foot clover	13
Verbascum thapsus L.	Mullein	24
Verbena urticaefolia L.	White vervain	15

[1] Reference is made by italic numbers in parentheses to literature citations (p. 439) in which reports of susceptibility were made.
[2] Inoculation only.

and after being grown on very highly infested soil, these species showed no indications of infection.

For certain of these species only a small number of plants were tested. However, extensive observations during the last four seasons on the weed floras of infested fields have been used to supplement the results obtained from the susceptibility tests.

DISCUSSION

The main interest of these experiments lies in the relation of the host range of *Bacterium solanacearum* to the control of Granville wilt by crop rotation. The results provide a basis for the experiments now in progress on the control value of various cultivated crops when used in rotations and on the effect of susceptible weeds on the degree of control obtained. Certain crop plants were not considered safe for use in rotations on infested land because they were included in the host list of *Bact. solanacearum*. Evidence of susceptibility lies in part in their susceptibility to inoculation. The results presented in table 2 and the illustrations in figures 4 and 5 show that some plants were positive to artificial stem inoculation and negative to natural infection.

If inoculation by artificial methods and subsequent reisolation of the parasite are accepted as adequate fulfillment of Koch's postulates, such plants must be considered as susceptible. However, the fact remains that they are not infected by the soil-borne organism when grown on very highly infested soil. It appears that when the organism is introduced through wounds into the stems of certain plants their mechanism of resistance is broken down. Such plants are susceptible as the result of artificial manipulation and hence are not susceptible to the disease in which primary invasion occurs through the roots from soil-borne infection. It is believed that these species should not be considered as host plants. The term "host plant" loses its practical significance if plants so listed do not develop the disease in the field and do not play an active part in the persistence and spread of the causal organism. This situation is similar to that found by Clayton (*2*), Johnson (*11*), Tisdale (*21*), and Young (*24*), who demonstrated that artificial methods might be misleading if exclusively used to determine host range of a number of parasites.

For the practical purposes of crop rotation, plants that are immune to natural infection should be safe for use on infested soil. Experiments by Garner, Wolf, and Moss (*7*) with cowpeas and experiments in progress by the author with cowpeas and soybeans show that rotations of these plants reduce the amount of wilt on succeeding crops of tobacco. Extensive farm experience corroborates these data.

SUMMARY AND RECOMMENDATIONS

The susceptibility of 90 plant species to *Bacterium solanacearum* E. F. Smith was established by (1) stem inoculation made with wedges of woody tissue from diseased tobacco plants, and (2) natural infection when grown in the field on naturally infested soil under very severe disease conditions. The species were divided into three classes on the basis of their reaction to these two tests: Class 1, susceptible to both stem inoculation and natural infection; class 2, susceptible to stem inoculation and immune to natural infection; class 3, immune under both methods of testing.

Class 1 includes 29 species. Of these tobacco, tomato, *Nicotiana rustica*, eggplant, jimsonweed, black nightshade, croton, Spanish-needles, 1 species of cocklebur, and nasturtium became 100-percent diseased.

Class 2 includes five species. Of these, cowpeas, soybeans, and velvetbeans were studied intensively because of their importance as field crops. A number of agronomic varieties of each were grown, and they remained immune under very severe disease conditions. All were susceptible after stem inoculation, but it was shown that the number of bacteria in the tissues of these plants was vastly smaller than the number present in infected tobacco. The organism was not able to multiply rapidly in the tissues of these plants. Since these species are immune to natural infection, it appears that they should not be listed as true hosts.

Class 3 includes 56 species that were immune to artificial inoculation and natural infection.

In conclusion, the following changes are recommended for the host list of *Bacterium solanacearum:*

(1) Remove from the host list: Sweetpotato, cotton, watermelon, fireweed, *Crotalaria striata*, velvetbeans, lima bean, soybeans, and cowpea.

(2) Add to the host list: *Xanthium pennsylvanicum* Wallr., *X. chinense* Mill., *Physalis pruinosa* L., *Aster pilosus* Willd., and *Ambrosia trifida* L.

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EFFECT OF TEMPERATURE ON SIZE OF EGGS FROM PULLETS IN DIFFERENT LATITUDES[1]

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THE PROBLEM

It has been shown by Bennion and Warren (*2*) [3] that high environmental temperatures materially influence the size of fowls' eggs. It is also well known that the eggs of pullets rapidly increase in size during the first few months of production. The few instances in which egg size has been recorded throughout the pullet year (*2, 5*) have shown the mean egg size to increase rapidly till early spring, after which it declined rather consistently throughout the summer. These records were taken in the central Temperate Zone.

The results of Bennion and Warren indicated that much of the reduction in egg size observed in late summer is associated with periods of excessively high temperature. In view of this fact it is of interest to compare the curves of annual egg size for pullets kept in different latitudes where the temperatures would vary greatly. It is not the purpose of this study to demonstrate the influence of temperature on egg size, since it is believed that this demonstration has been more convincingly made under controlled conditions (*2*). It is desired here to show how the curves of annual egg size may vary when observations are made under different temperature conditions.

MATERIALS AND METHODS

In order to secure data for the construction of annual egg-size curves for birds kept in various latitudes, records were obtained from scientific workers in different parts of continental United States, the Philippine Islands, Canada, and Scotland. The data used were secured from the records of agricultural colleges or from recognized egg-laying contests. Further information regarding the data is given in table 1.

In all instances daily egg-size records from individual birds were secured and from them mean daily egg size was calculated by the author. The minimum number of birds used for constructing a curve was 25 and the maximum 146. In all instances only the records of those pullets that laid fairly consistently over the entire period under consideration were used in the calculation, thus reducing the possibility of not having a uniform sample throughout the year. Various units of measure of egg size were found in the original data, but for all the curves constructed, the results were converted into weight in grams. Temperature was expressed in degrees Fahrenheit for the corresponding year. In some cases the original units of

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[3] Italic numbers in parentheses refer to Literature Cited, p. 452.

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measure of egg size were not so small as desired, but since the points on the curves were for means calculated from several records it is believed that the results are dependable. In all of the work any eggs that appeared to be yolkless or double yolked were eliminated from consideration. Since individual egg records were available, such abnormal eggs could usually be recognized. In the original summarization, curves were made for daily maximum temperatures and daily mean egg weights for each region, but to conserve space most of the curves presented were constructed from the mean of semimonthly periods calculated from the daily means. In each of the curves of egg size shown, horizontal lines are drawn at the 56-gm. weight and the 70° temperature for purposes of comparison. These two points were chosen because 56 gm. probably represents the ideal for egg weight and 70° F. seemed to be the minimum at which a pronounced effect was shown by egg size. It is the shape and not the level of the egg-weight curves that is of interest. The mean egg size for any group is probably an expression of inherent qualities that are not here considered.

TABLE 1.—*Sources of data used in this study*

Location	Degrees north latitude	Range of daily maximum temperature	Breed of chicken	Type of records	Year
		°F.			
Roslin, Scotland	56	32 to 76	White Wyandotte White Leghorn	Contest	1935-36
Charlottetown, Prince Edward Island, Canada	46	−1 to 82 5 to 87	Barred Plymouth Rock	do do	1928-29 1930-31
Nappan, Nova Scotia, Canada	46	−7 to 84	White Leghorn Barred Plymouth Rock	do	1924-25
Macdonald College, Quebec, Canada	45	−4 to 88	White Leghorn	College	1927-28
Amherst, Mass	42	12 to 96	Rhode Island Red	do	1935-36
East Lansing, Mich	43	3 to 106	White Leghorn Barred Plymouth Rock	Contest	1934-35
Manhattan, Kans	39	13 to 110 4 to 116	White Leghorn Crossbred	College	1921-22 1935-36
Davis, Calif	38	47 to 110	White Leghorn	do	1934-35
College Station, Tex	31	41 to 109	do	do	1933-34
Chipley, Fla	31	35 to 97	do	Contest	1934-35
Los Baños, Laguna, P. I	14	77 to 96	do	College	1934-35

MID-TEMPERATE ZONE DATA

The data collected in the mid-Temperate Zone should best show the effects of temperature on egg size since in this zone the greatest extremes in temperatures are found. Considerable data are available from Manhattan, Kans., one set of which is shown in figure 1. In this figure the relationship of the curves of daily maximum and of daily mean egg weight are shown for a group of 146 crossbred pullets mostly from the Black Minorca White × Leghorn cross. It is seen that low winter temperature fluctuations have no influence on egg size, since the curve of egg size consistently rises until sometime in February. This rise is characteristic of pullets during the initial stages of egg production. After reaching the high point about the middle of February the egg-size curve begins to recede. From that time on, the curves of egg size and temperature are excellent counterparts of each other, the high points in the temperature curve being associated with points of small egg size. As will be shown in other

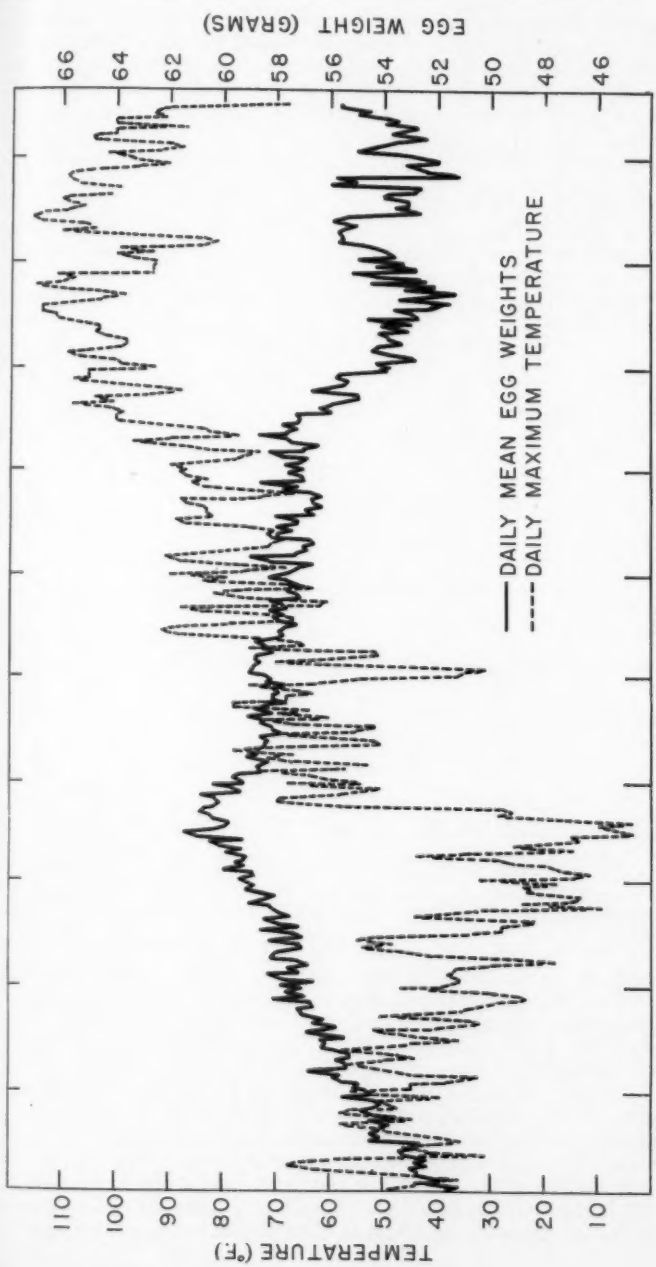

FIGURE 1.—Relation of curves of daily maximum air temperatures and daily mean egg weights for a group of 146 crossbred pullets kept at Manhattan, Kans., 1935–36.

graphs, when the maximum temperature consistently remains above 70° F. the egg size begins to decline, reflecting in inverse proportion the fluctuations in temperature. Any extreme changes in temperature extending over periods of a few days are reflected in fluctuations in egg size. From these results it would seem that much of the decline in egg size during the summer season at Manhattan, Kans., is due to the high temperatures of that section. This opinion is strongly confirmed by the results of Bennion and Warren (*2*) secured under controlled temperature conditions.

In the graphs showing egg size from various latitudes the data have been averaged for semimonthly periods. Although this tends to obscure the short period effects it shows to better advantage the annual trends. Figure 2, *A*, gives the data for a group of White Leghorns

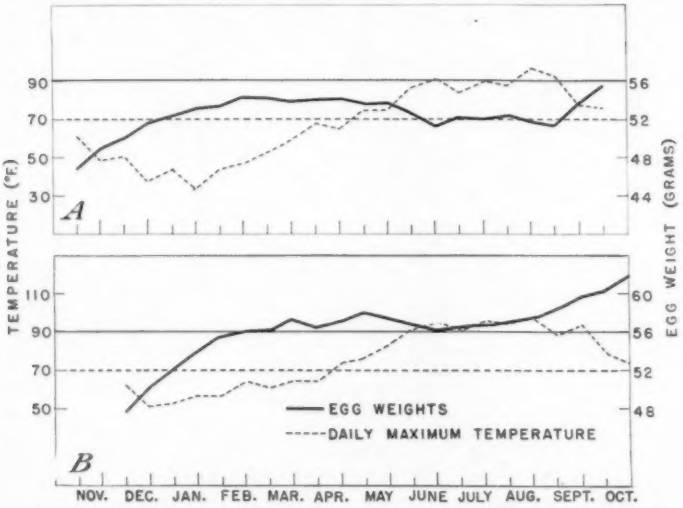

FIGURE 2.—Mean maximum air temperatures and mean egg weights at semimonthly periods for groups of pullets kept in the mid-temperate zone, showing the influence of temperature on annual egg-size curves: *A*, 62 White Leghorns at Manhattan, Kans., 1921–22; *B*, 40 White Leghorns at Davis, Calif., 1934–35.

at Manhattan, Kans., for the year 1921–22. The trends are very similar to those of the crossbreds (fig. 1) except that a greater number of extremely high temperatures were encountered in 1935–36.

Figure 2, *B*, was constructed from data taken at Davis, Calif., which lies in approximately the same latitude as Manhattan, Kans. At Davis in 1935 a temperature above 70° F. was reached somewhat earlier than in the 1922 Kansas data. Figure 2 A, B, shows very similar summer maximum temperatures. The California egg-weight curve is similar to the Kansas one except that there is less depression of the curve in summer. A possible contributing factor not shown in the figures is the daily minimums. Those of California ranged from 10° to 18° lower than the minimums for Kansas. The cooler nights

may have afforded an opportunity for some recovery from the effects of the heat in California and so have accounted for less depression of the egg-weight curve. It is of interest to note the sharp increase of the California egg size in August and September following the rapid decline in temperatures at that season.

TROPICAL AND SUBTROPICAL DATA

Data were available for points in tropical and subtropical regions, including College Station, Tex.; Chipley, Fla.; and Los Banos, Laguna,

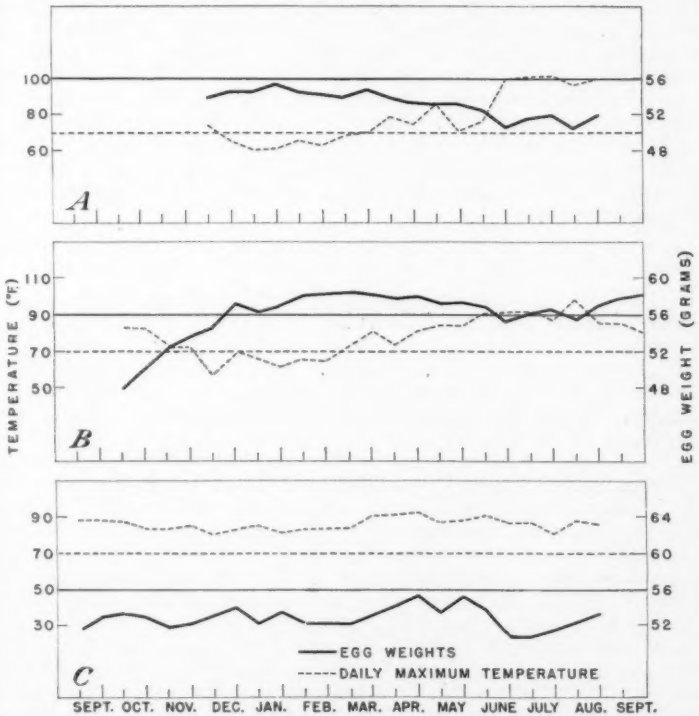

FIGURE 3.—Mean maximum air temperatures and mean egg weights at semi-monthly periods for groups of pullets kept in tropical and subtropical regions, showing the influence of temperature on annual egg-size curves: *A*, 45 White Leghorns at College Station, Tex., 1933–34; *B*, 51 White Leghorns at Chipley, Fla., 1934–35; *C*, 30 White Leghorns at Los Banos, Laguna, P. I., 1934–35.

P. I. (fig. 3). The curves from these regions are not strictly comparable with those from more northerly latitudes since the maximum temperatures for much of the winter season in the southern latitudes are near or above the 70° minimum at which temperatures are noticeably effective in reducing egg size. Thus the curve of egg size would tend to have its peak flattened, making the depression at the period of highest temperature appear less extreme. Such a condition is

evident in the Texas and Florida curves. The increase in egg size at the very end of the laying year, accompanied by a decline in temperature, is shown in the Florida data.

An interesting curve of annual egg size was secured from the data recorded at the agricultural college at Los Banos, Laguna, P. I. The location is near the Equator and the maximum temperatures are uniformly high throughout the year. From table 1 it will be seen that the extreme range of maximum temperature is 77° to 96° F. When averaged for 15-day periods as shown in figure 3, *C*, the temperature curve is almost a straight line at about 86°. The form of the curve of mean egg size differs considerably from those constructed from other data. Although there are fluctuations in egg size the trend approaches a horizontal line. Doubtless the uniformly high temperatures have suppressed the egg size throughout the entire year, thus preventing the attainment of a size that would have been possible under lower temperatures. There is an increase in egg size in April and May and a decline in June which have no accompanying variation in temperature. There was a marked increase in rainfall at about the time of the June decline, but it is not known whether this had a depressing effect.

NORTHERN LATITUDE DATA

In the more northerly latitudes data were available from East Lansing, Mich.; Amherst, Mass.; Macdonald College, Quebec, Canada; Charlottetown, Prince Edward Island, Canada; Nappan, Nova Scotia, Canada; and Roslin, Scotland. Conditions in these regions as they affect egg size are markedly different from those in the southern latitudes. In some of the northern localities the entire range of temperature is below the point at which egg size is noticeably affected by temperature, while in some of the southern localities from which data were obtained the entire temperature range was above this point.

In Massachusetts and Michigan (fig. 4) the extreme of high temperatures sometimes approached those reached in Kansas, but the periods of high temperature were less sustained and the daily minimum temperatures were considerably lower. The curves of egg size from Michigan and Massachusetts each showed a slight depression during July and August when the temperatures reached a maximum. However, the form of the curve is very different from that secured at Manhattan, Kans. The curve for East Lansing, Mich., follows nearly a straight line after reaching a maximum in February. The records from Michigan included data on both White Leghorns and Barred Plymouth Rocks, and it is to be noted that the curves for two breeds are almost identical.

The Canadian data tend to confirm the results from the northern United States, indicating that where the maximum temperature seldom exceeds 70° F. for periods of any length, the egg size is little depressed. Data were summarized from White Leghorns at Macdonald College, Quebec, and Barred Plymouth Rocks at Nappan, Nova Scotia, and Charlottetown, Prince Edward Island, there being results for 2 different years at Charlottetown. These data are presented in figures 5 and 6. Although there is some variation among the curves, the results are in close agreement in showing little or no

depression of egg size during the summer. The general form of the curves shows a gradual rise during the entire pullet year, although the slope is rather slight after February. The maximum temperature curve seldom exceeds 70° for the Canadian data and then remains so only briefly. These data indicate that where high temperatures do not interfere, there is an increase in egg size throughout the pullet year.

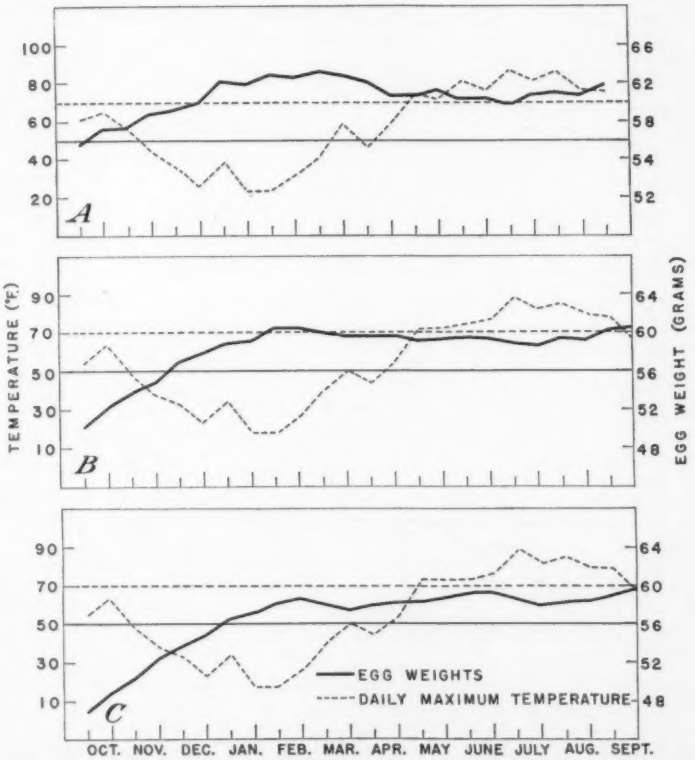

FIGURE 4.—Mean maximum air temperatures and mean egg weights at semi-monthly periods for groups of pullets kept in northern latitudes, showing the influence of temperature on annual egg-size curves: *A*, 25 Rhode Island Reds at Amherst, Mass., 1935–36; *B*, 106 White Leghorns at East Lansing, Mich., 1935–36; *C*, 61 Barred Plymouth Rocks at East Lansing, Mich., 1935–36.

Data were secured on White Wyandottes and White Leghorns at Roslin, Scotland. The curves in figure 7 for the two breeds during the same year are in close agreement. The mean maximum temperatures by 15-day periods never exceeded 70° F., although the daily maximum did reach 76°. The results here agree with those from the Canadian data in showing a maximum of egg size at the period of maximum temperatures during August and September. There was,

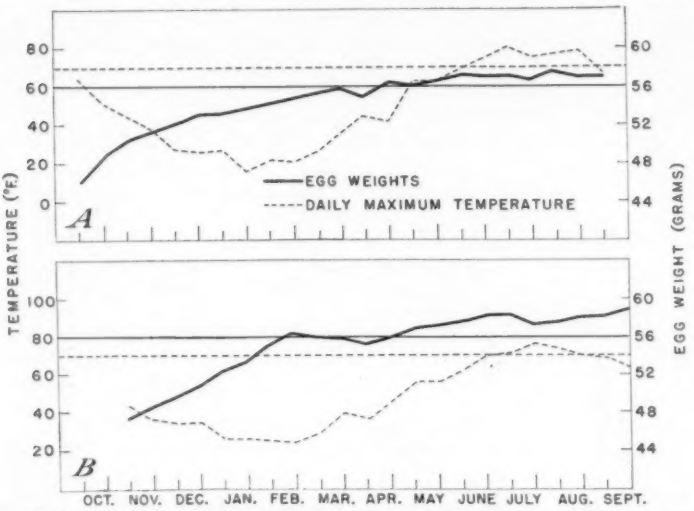

FIGURE 5.—Mean maximum air temperatures and mean egg weights at semi-monthly periods for groups of pullets kept in northern latitudes, showing the influence of temperature on annual egg-size curves: *A*, 83 White Leghorns at Macdonald College, Quebec, Canada, 1927–28; *B*, 76 Barred Plymouth Rocks at Charlottetown, Prince Edward Island, Canada, 1928–29.

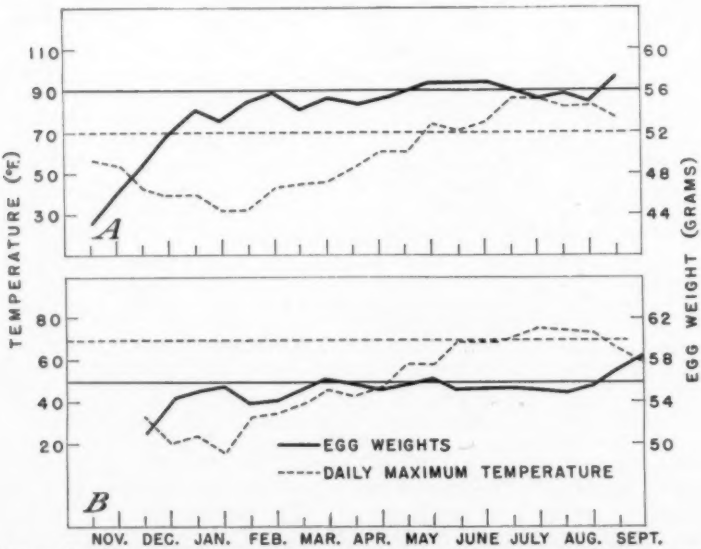

FIGURE 6.—Mean maximum air temperatures and mean egg weights at semi-monthly periods for groups of pullets kept in northern latitudes, showing the influence of temperature on annual egg-size curves: *A*, 59 Barred Plymouth Rocks at Charlottetown, Prince Edward Island, Canada, 1930–31; *B*, 107 Barred Plymouth Rocks at Nappan, Nova Scotia, Canada, 1924–25.

however, an unexplainable decline in egg size during May and June when the temperature is not high enough to influence egg size. The results in this period are so far out of line with other data as to suggest the influence of some unidentified environmental factor.

DISCUSSION

From the data presented in this and earlier publications it seems fairly evident that egg size is materially reduced by high temperatures. It is recognized that the curve of annual egg size for any locality is representative of the effects of temperature for the particular year recorded and that the curve might vary considerably under a different set of conditions. The use of maximum temperatures rather than the mean daily temperatures might be questioned, since the relief which the bird gets at night is probably a contributing factor

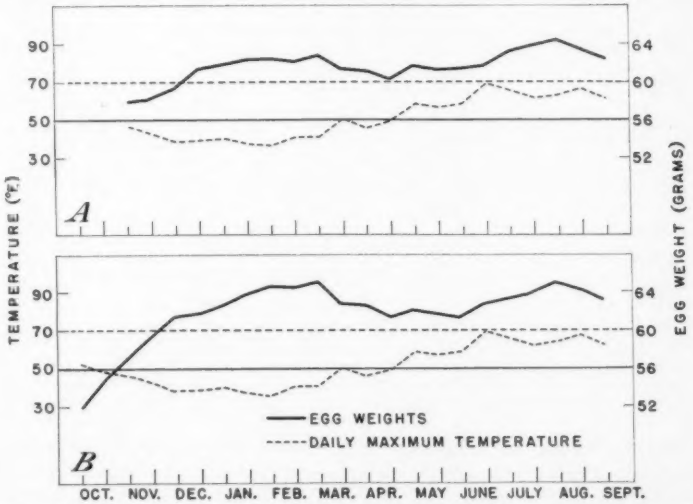

FIGURE 7.—Mean maximum air temperatures and mean egg weights at semi-monthly periods for groups of pullets at Roslin, Scotland, 1935–36, showing the influence of temperature on annual egg-size curves: *A*, 42 White Wyandottes; *B*, 41 White Leghorns.

in the reaction of her egg size to temperatures. However, the extreme height to which the day temperatures go is also an important factor which would be somewhat masked by the daily mean. Where data were available for more than one breed for the same year and locality, the curves were very similar, indicating that the available samples were representative and that many of the minor fluctuations in egg size are the result of unidentified environmental influences. Low winter temperatures seem to have no influence on egg size.

No explanation is offered as to the manner in which high temperatures produce their effect. Galpin (*4*) attempts to explain seasonal changes in egg size by attributing them to variations in the metabolic

level of the hen. He finds a paralellism between seasonal curves of thyroid weight and egg weight. Riddle (*7*) found a similar seasonal variation in thyroid weight in pigeons which he believed to be a response to external temperature variations. Asmundson and Pinsky (*1*) found that the feeding of thyroxin decreased egg size. These results as a whole suggest a relationship between egg size and the functioning of the thyroid which in turn may be conditioned by external temperature variations. The problem of how the three factors are related requires further investigation. The study of Bennion and Warren (*2*) showed that all parts of the eggs were reduced in weight but that the percentage decrease was greatest in the shell and egg white. This would indicate that the functioning of the oviduct is more affected than that of the ovary.

From the data here presented it is difficult to state the exact minimum at which temperature becomes a factor in influencing egg size. These data are from the pullet year and there is evidence of an inherent

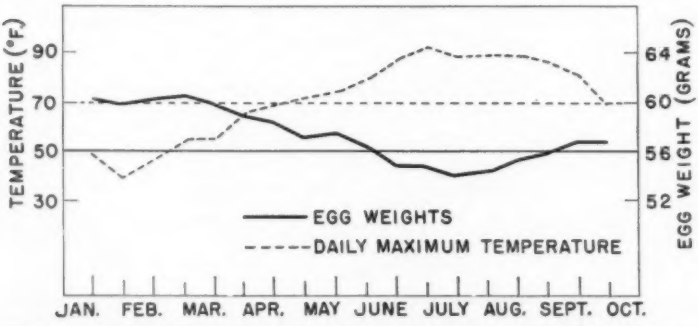

FIGURE 8.—Mean maximum air temperatures and mean egg weights at semimonthly intervals for 20 White Leghorn hens (second-year) at Manhattan, Kans., showing the influence of temperature on annual egg-size curves, 1923–24.

tendency of eggs to increase in size during the year. If pullets encounter temperatures high enough to depress egg size, the antagonistic influences will neutralize one another and the minimum point at which temperatures are effective will be obscured. Only when the temperatures are high enough to counteract the tendency for eggs to become larger as the year progresses, will the influence of high temperature be evident. For this reason data on pullets are not very crucial in determining the minimum at which temperatures are effective in depressing egg size. Results from hens in their second or later laying years would be more exact for this purpose. In figure 8 the data from hens do indicate that egg size is depressed by temperatures as low as 50° F. Most of the curves involving pullet data do not show much effect of temperature at this low point. In many of the curves presented in this paper it is evident that when the maximum temperature exceeds 70° for very many days there is a depressing effect on the egg size. There are other instances in which it appears that sudden increases in temperature at 40° and 50° may temporarily suppress the size of the egg. Precipitous changes in temperature, within the effective range, seemed to have a more pronounced imfluence on egg

size. Lorenz and Almquist (*6*) found evidence of a decrease in egg weight proportional to the increase in temperature between 40° and 90°. Bruckner (*3*) found that under controlled conditions in a house where the temperature was held at a mean of 50° there was evidence of a depressing effect on egg size in winter as compared with the egg size of birds subjected to the normal temperatures of New York.

The fact that egg size is so sensitive to changes in environmental temperatures has a significant bearing on the problem of what constitutes an accurate measure of egg size. If records for short periods are to utilized, the temperature encountered during those periods must be taken into consideration. Even if the mean egg size for the entire pullet year is used as a measure, the worker is confronted with the temperature problem in comparing one year's results with another. This problem is also encountered in selection studies in comparing dam and daughter egg size, and it is a factor that cannot be ignored in genetic studies where succeeding generations must be compared.

The question may raised as to what constitutes a normal curve of egg size for the pullet year. An examination of results secured in the more northern latitudes indicates that if high temperatures do not interfere, egg size continues to increase throughout the entire first year of production. If this be true then pullets kept in the more southern latitudes never reach their maximum potential egg size. This has a very definite bearing upon any Nation-wide breeding program in which birds from different regions are compared for egg size.

The fact that egg size usually declines during the summer in many regions is a matter of common knowledge, and the opinion has sometimes been held that the decline is the expression of the fatiguing influence of a long period of production. The lack of any decline in egg size near the end of the laying year in the data from northern latitudes, however, does not support this view. Even more convincing refutation is seen in the curves which show a depressing effect of summer temperatures. In such instances, there was usually a rapid increase in egg size at the very end of the pullet year, provided the temperature decreased significantly. If fatigue were a factor in producing small egg size in summer then the recovery at the very end of the laying year would not be expected.

In order to determine whether the depressing effects of high temperature on egg weight may be found in the eggs of hens as well as pullets, data were summarized from the second-year records of a group of White Leghorns. Although the data are rather limited the form of the curve (fig. 8) is quite similar to that of pullets from the same latitude, except that egg size for the older birds started at a point near the maximum for the pullet year. There was the same striking summer depression in egg size that characterized the data from pullets.

White Leghorns, Barred Plymouth Rocks, Rhode Island Reds, White Wyandottes, and crossbreds were included among the breeds investigated, and it would seem, therefore, that the results secured would apply generally.

There were a few instances of depression of egg size with which change in temperature could not be associated. This would indicate that factors of the environment other than temperature may influence egg size. It is improbable that temperature, although important, is the only environmental factor that conditions egg size. It is also

possible that some instances of unaccounted-for variation in egg weight might have been due to the fact that the weighing devices were temporarily out of adjustment.

Although the data here presented are all in the form of means, several instances were observed in which individuals appeared to vary in their susceptibility to the depressing influence of high temperatures. This would suggest the possibility that birds might be bred for ability to continue with satisfactory egg size when subjected to adverse temperature conditions.

SUMMARY

Data were secured on egg size from 11 localities in latitudes extending from the Equator to as far north as Scotland. Curves showing mean daily egg weights together with the daily maximum temperatures for each locality were drawn for the pullet laying year. A comparison of the various curves of annual egg size showed that temperature was a very important factor in determining the shape of the curve. In most instances egg size increased rapidly for the first few months as a result of approaching physiological maturity. Following this, egg size was found to bear a close relationship to the temperatures prevailing. Excessively low temperatures seemed to have no effect on egg size, but after the daily maximum temperatures exceeded 70° F. for a period of a few days or more, egg-size fluctuations usually showed a close correlation with those of temperature. Where the maximum temperature seldom exceeded 70° egg size increased throughout the entire pullet year, with the rate of increase relatively slow in the last half of the year. In localities where summer temperatures were high, the summer egg size was greatly depressed, and the bird was prevented from expressing its maximum potentialities for egg size unless the temperature dropped below 70° at the end of the laying year. If this decline in temperature occurred, the egg size increased rapidly thus indicating that the summer decline was not the result of any fatiguing effect of a long period of production.

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VIABILITY OF POLLEN AND OVULES OF BARLEY AFTER COLD STORAGE [1]

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INTRODUCTION

The pollen of many plants can be kept viable during shipment or storage for the pollination of later maturing varieties. However, no very satisfactory method has been found for storing pollen of barley (*Hordeum* spp.), because of its great sensitivity to variations in relative humidity. The pistil of barley, on the other hand, is much less easily injured.

This study of the effect of various periods of cold storage upon the reproductive processes of the barley plant was made to determine (1) seed setting in unemasculated spikes allowed to resume activity after cold storage, (2) the viability of pollen stored in unemasculated spikes, and (3) the seed productivity of ovules in stored emasculated spikes. A set of seed involves not only the retention of viability in the pollen and ovules but also the dehiscence of anthers and accompanying pollination of stigmas after storage.

MATERIALS AND METHODS

The barley varieties utilized in this study (table 1) were grown during the winter and spring of 1937–38 at the Arlington Experiment Farm, Arlington, Va., in the greenhouse or in field plots.

TABLE 1.—*Barley varieties grown at Arlington, Va., during winter and spring of 1937–38*

Variety	C. I.[1] No.	Description
Hannchen	531	2-rowed awned, spring.
Spartan	5027	Do.
Wisconsin Pedigree 38	5105	6-rowed awned, spring.
Smooth Awn 86	6268	6-rowed awned, winter.
Beardless 6	2746	6-rowed hooded, winter.
Hooded 6	6270	Do.
Brugh 76	6477	Do.

[1] Accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

Spikes at stages before anther dehiscence were prepared for emasculation by clipping off the distal portion of the boot leaf just below the spike, removing the lateral florets, and cutting off the lemmas and palets of the central florets just above the distal tips of the anthers. They were then emasculated and bagged as for hybridization, except that a slightly greater range of maturity was included. One to five days later, when the flowers exhibited a rather wide range of receptivity as judged by the spread of the lemma and palet and extrusion

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of the stigma, the culms bearing these emasculated spikes were clipped off above the third node below the spike, so as to include the remnant of the boot and the entire leaf just below it. The cut ends were then placed in flasks containing tap water, the spikes being protected from excessive dehydration by glassine bags, and put into dark cold storage. With each lot of emasculated spikes were stored culms bearing unemasculated spikes with undehisced anthers, as a source of stored pollen.

The spikes from greenhouse-grown Hannchen barley were held in refrigerated chambers at 36° and 40° F., while those from the remaining varieties grown in field plots were stored at 36°, 40°, and 50°. All three chambers were dark and were maintained at a relative humidity of about 82 percent. Fresh pollen and ovules from later plants of the same variety were available throughout most of the experiments; but for testing the viability of stored pollen and ovules in the field-grown material the supply was supplemented subsequently with spikes from later maturing plots on account of the rapid exhaustion of viable pollen and productive ovules. Toward the end of the experiment fresh pollen from the two spring-sown varieties, Spartan and Wisconsin Pedigree 38, was used.

To test the ability of unemasculated stored spikes to set seed, a few were selected at random and placed in Erlenmeyer flasks containing tap water.

For testing stored pollen in the greenhouse experiment, fresh unpollinated spikes were always available, and flowers on these were dusted with stored pollen and allowed to mature seed upon the plant. For testing stored field pollen it was necessary to use fresh or stored spikes that had been cut from the plant. Since much of the stored pollen did not dehisce readily, the procedure of dusting the same flower with pollen from several spikes was followed. The spikes dusted with stored pollen were then placed in tap water and allowed to develop.

In testing the productivity of ovules of stored emasculated spikes, a number of spikes were chosen that exhibited as wide a range of flower receptivity as seemed desirable. These were pollinated with fresh pollen, when available, or with the pollen from the unemasculated spikes held in cold storage. The pollinated spikes were protected with glassine bags and likewise placed in flasks containing tap water. All the flasks containing the excised greenhouse spikes were then set on a well-lighted greenhouse bench, and those containing the field-plot spikes were staked upright in a bed in a nursery cage outside and allowed to resume activity. When the culms had largely lost their chlorophyll and seed development had apparently ceased, the spikes were removed to the laboratory, placed in paper envelopes, and dried.

EXPERIMENTAL RESULTS

SEED PRODUCTION OF STORED UNEMASCULATED SPIKES

Table 2 gives the seed set on the unemasculated spikes of greenhouse-grown plants held in tap water under favorable growing conditions after four periods of storage. Few seeds were set, but no spike failed entirely except the two stored for 21 days at 40° F.

In the field-grown barley (table 3), 29 days of storage at 36° permitted the development of 7 seeds in 1 out of 8 spikes. One seed developed in 1 out of 9 spikes that had been kept at a temperature of

40° for 26 days, and 10 seeds were found in 2 of the 12 spikes that had been stored 14 days at 50°. Photographs of the field-grown seeds are shown in figure 1.

TABLE 2.—*Seed set on unemasculated spikes of greenhouse-grown barley, grown in tap water after storage at 36° and 40° F.*

Storage period (days)	36° F.		40° F.		Storage period (days)	36° F.		40° F.	
	Spikes	Seed set	Spikes	Seed set		Spikes	Seed set	Spikes	Seed set
	Number	*Number*	*Number*	*Number*		*Number*	*Number*	*Number*	*Number*
9	5	15	5	7	16	3	11	3	9
13	5	14	5	9	21	3	3	2	0

TABLE 3.—*Seed set on unemasculated spikes of field-grown barley, grown in tap water after storage at 36°, 40°, and 50° F.*

Storage period (days)	36° F.			40° F.			50° F.		
	Spikes		Seeds	Spikes		Seeds	Spikes		Seeds
	Total	Fertile		Total	Fertile		Total	Fertile	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
14	11	11	(1)	12	5	22	12	2	10
19	9	2	5	8	0	0	7	0	0
26	9	0	0	9	1	1	----	----	----
29	8	1	7	6	0	0	----	----	----
31	----	----	----	----	----	----	----	----	----
35	5	0	0	7	0	0	----	----	----
39	12	0	0	12	0	0	----	----	----
42	----	----	----	----	----	----	----	----	----

[1] Many seeds.

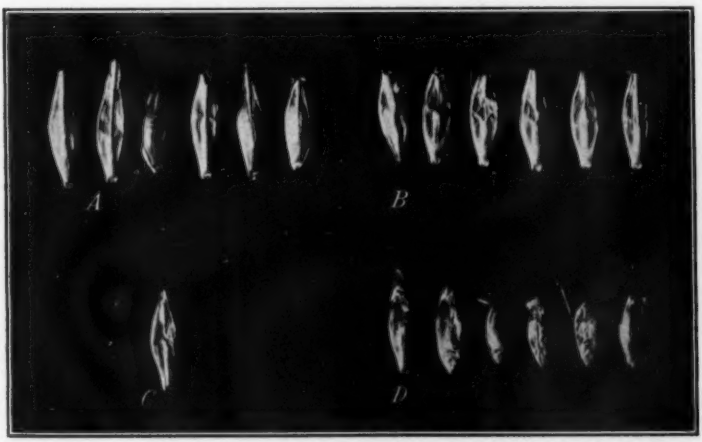

FIGURE 1.—*A*, Field-grown Beardless 6 barley matured on the plant. (Germination test in row 10, fig. 3.) *B*, Seed from unemasculated spikes stored 29 days at 36° F. (Germination test in row 11, fig. 3.) *C*, Seed from unemasculated spike stored 26 days at 40°. (Germination test in row 12, fig. 3.) *D*, Seed from unemasculated spike stored 14 days at 50°, badly moth-eaten. (One seed produced the plant shown in row 13, fig. 3.)

Unemasculated spikes from the greenhouse gave some indications of growth during storage. Two days after the culms were put into the chambers, the awn tips had extruded from the boot about 10 mm., and after 3 weeks the maximum growth measured was about 15 mm. Normal extrusion of the awn of Hannchen in the greenhouse was 23.5 mm. in 24 hours.[2] The flowers opened during storage if they were sufficiently mature when placed in storage, but only an occasional spike showed elongation of the anther filament. Only a small proportion of the spikes were harvested at the proper stage to produce dry pollen in ripe anthers immediately after removal from cold storage.

VIABILITY OF POLLEN IN REFRIGERATED BARLEY SPIKES

The viability of pollen from the spikes grown in the greenhouse was tested, after five periods of storage in the spike, on receptive flowers of growing greenhouse plants. In table 4 it is seen that pollen stored for 21 days at 36° F. was still able to produce seed, while that stored for the same length of time at 40° failed entirely. Nine days of storage gave a very poor seed set at 36° and none at 40°. This pollen, when germinated on receptive flowers and examined, showed a viability of 57 percent for storage at 36° and but 29 percent for storage at 40°, with many of the pollen tubes growing weakly and abnormally.

TABLE 4.—*Seed set by pollen from spikes of greenhouse-grown barley, stored at 36° and 40° F., upon flowers of receptive greenhouse plants*

Storage period of pollen (days)	36° F.				40° F.			
	Spikes	Flowers	Seeds	Seed set	Spikes	Flowers	Seeds	Seed set
	Number	*Number*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Percent*
6	1	24	16	66.7	2	42	3	7.1
9	1	19	1	5.3	1	21	0	0
13	3	49	48	98.0	3	48	44	91.7
16	3	27	19	70.4	3	42	4	9.5
21	3	26	5	19.2	3	18	0	0

For testing the pollen of refrigerated field-grown culms it was necessary in most cases to use emasculated spikes that also had been stored. The period of storage in each case is indicated in table 5. At 36° F., pollen stored 26 days produced one seed in a spike that had been stored 15 days and three seeds in a spike stored for 26 days (fig. 2, *A*). This pollen was noted as "good." After this date the pollen deteriorated rapidly, and ovules stored longer than 26 days were very poor as pollen testers.

At 40° F., 1 spike stored 19 days had anthers dehiscing sticky pollen, which produced 4 seeds on 20 flowers stored 19 days at 36° (fig. 2, *B*). Pollen spikes stored 10 days at 50° were flowering, but the anthers were still striped with green, while on the fourteenth day the flowers were generally open, with anthers extruded and pollen scarce. No seeds set on 18 flowers. Dry pollen was noted on the nineteenth day, but no seeds were produced.

A good seed set resulted from pollen and ovules, both stored 19 days at 36° F. The longest successful pollen storage at 36° was 26 days, and at 40° it was 19 days. No seed was obtained from pollen stored at 50°.

[2] Unpublished 1920 data.

FIGURE 2.—*A*, Spike showing 3 seeds produced by pollen stored 26 days at 36° F. upon ovules stored 26 days at 36°. (Germination test in row 14, fig. 3.) *B*, Spike showing 4 seeds produced by pollen stored 19 days at 40° upon ovules stored 19 days at 36°. (Germination test in row 15, fig. 3.) *C*, Emasculated spike stored 42 days at 36°, which set 5 lateral seeds after being dusted with fresh pollen. (Germination test in row 16, fig. 3.) *D*, Emasculated spike stored 29 days at 40°, which set 1 seed after being dusted with pollen stored 18 days at 36°. (Did not germinate.) *E*, Emasculated spike stored 14 days at 50°, which set 1 seed after being dusted with fresh pollen. (Germination test in row 17, fig. 3.)

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TABLE 5.—*Seed set by pollen from spikes of field-grown barley stored at 36°, 40°, and 50° F., upon best available ovules*

Storage period of pollen (days)	36° F.						40° F.					50° F.[1] pollination[2]	
	Pollination			Seed set			Pollination[1]		Seed set				
	Spikes	Ovules stored	Flowers	Spikes fertile	Seeds	Percent	Spikes	Flowers	Spikes fertile	Seeds	Percent	Spikes	Flowers
	No.	*Days*	*No.*	*No.*	*No.*		*No.*	*No.*	*No.*	*No.*		*No.*	*No.*
8	2	19	36	2	28	77.8							
14	1	0	20	1	7	35.0	1	21	1	6	28.6	1	18
15	3	26	48	3	11	22.9							
16	1	35	18	0	0	0							
18	3	29	41	1	1	2.4							
19	2	19	42	2	17	40.5	1	20	1	4	20.0	1	17
20	1	39	18	0	0	0							
24	3	35	56	0	0	0							
26	1	15	19	1	1	9.5	1	15	0	0	0		
	1	26	23	1	3								
29	2	29	36	0	0	0	1	23	0	0	0		
35	1	35	19	0	0	0	1	17	0	0	0		
39							1	19	0	0	0		

[1] No seed set at 50° F.
[2] Ovules same age as pollen but stored at 36° F.

SEED PRODUCTION AFTER COLD STORAGE OF EMASCULATED SPIKES

As would be expected, growth and development in emasculated spikes were greatly slowed down in cold storage, the normal cycle of flower opening and gradual closing proceeding in a much more leisurely manner. During the later storage stages the stigma hairs appeared withered at the tips but looked normal throughout most of their length.

Fifty spikes of greenhouse-grown Hannchen barley, emasculated during the period from March 9 to 13, were cut off and stored on March 14, 25 being stored at 36°, and 25 at 40° F. Spikes were moved at intervals from the refrigerator to the greenhouse, where they were dusted with fresh pollen and left with cut ends in tap water, on a well-lighted bench. Table 6 shows the percentage of seed set in emasculated spikes stored 7 to 21 days before pollination.

TABLE 6.—*Seed set on emasculated spikes of greenhouse-grown barley when pollinated with fresh greenhouse pollen after cold storage*

Storage period (days)	36° F.					40° F.				
	Pollination		Seed set			Pollination		Seed set		
	Spikes	Flowers	Spikes fertile	Seeds	Percent	Spikes	Flowers	Spikes fertile	Seeds	Percent
	Number	*Number*	*Number*	*Number*		*Number*	*Number*	*Number*	*Number*	
7	4	81	3	29	35.8	5	107	1	1	0.9
10	5	108	5	83	76.8	5	114	4	66	57.9
14	5	111	4	38	34.2	5	114	5	56	49.1
17	5	124	3	28	22.6	5	110	3	24	21.8
21	6	133	2	11	8.3	5	114	2	15	13.2

An examination of the dates of emasculation of the 50 greenhouse grown spikes showed that by far the best seed set was obtained when refrigeration was begun 3 days or less after emasculation. Consequently, late in April, 125 spikes of Beardless 6 barley were emasculated during a 3-day period, divided into three lots, and stored at 36°, 40°, and 50° F. Unemasculated spikes as a source of pollen were stored with each lot from the following varieties: Beardless 6, at the same time; Smooth Awn 86 and Brugh 76, 11 days later; and Hooded 6, 19 days later. Nursery-grown Spartan and Wisconsin Pedigree 38 supplied the fresh pollen used toward the end of the experiment. Table 7 shows the seed set upon stored spikes dusted with the best pollen available.

TABLE 7.—*Seed set in emasculated spikes of field-grown barley after cold storage at 36°, 40°, and 50° F., by pollen fresh or stored at 36° F.*

Storage period of ovules (days)	36° F.						40° F.						50° F.					
	Pollination			Seed set			Pollination			Seed set			Pollination			Seed set		
	Spikes	Pollen stored	Flowers	Spikes fertile	Seeds	Percent	Spikes	Pollen stored	Flowers	Spikes fertile	Seeds	Percent	Spikes	Pollen stored	Flowers	Spikes fertile	Seeds	Percent
	No.	*Days*	*No.*	*No.*	*No.*		*No.*	*Days*	*No.*	*No.*	*No.*		*No.*	*Days*	*No.*	*No.*	*No.*	
8	1	19	21	1	12	57.1												
10	1	29	14	0	0	0												
14	2	0	37	2	25	67.6	5	3	98	5	50	51.0	5	3	93	1	1	1.1
15	1	26	19	1	1	5.3												
19	2	8	36	2	28	77.8	4	8	66	1	3	4.5	8	8	156	0	0	0
26	3	15	48	3	11	22.9	5	15	105	2	3	2.9						
29	3	18	41	1	1	2.4	5	18	80	1	1	1.3						
31	8	0	91	4	19	20.9	4	0	78	0	0	0						
35	1	16	18	0	0	0	4	16	70	0	0	0						
39	2	0	29	0	0	0	5	0	82	0	0	0						
42	4	0	109	1	5	4.6												

Under storage at 36° F. the flowers of emasculated spikes opened and finally closed, seemingly in a perfectly normal manner, but at a much slower rate than under field conditions. At 40° flower opening was much more rapid and all flowers were closed on the thirty-ninth day of storage, while at 50° the flowers had opened and nearly all were closed by the fourteenth day of storage.

The foliage color of the culms stored at 36° F. was well preserved after 42 days of storage, but at 40° the leaves had begun to yellow after 19 days, and at 50° both spikes and leaves were quite yellow on the nineteenth day and chlorotic by the twenty-sixth day.

Fresh pollen was used on the ovules stored 14, 31, 39, and 42 days, with a generally decreasing seed set, as would be expected. Likewise, the productiveness of ovules stored at 36° F. varied roughly with the freshness of the pollen used. Fresh pollen on the fourteenth day gave a higher percentage of seed set than 19-day pollen on the ovules stored 8 days; the 10-day ovules dusted with pollen stored 29 days yielded nothing, while the 15-day ovules gave a 5.3 percent seed set with pollen stored 26 days. An exceptionally high seed set of 77.8 percent was obtained with ovules stored 19 days and dusted with 8-day pollen. A considerable number of flowers seemed quite unaffected by excision and storage for nearly a month. On the twenty-sixth day the seed

set of the spikes stored at 36° was 22.9 percent, but it dropped to 2.4 percent on the twenty-ninth day. However, with fresh pollen on ovules stored 31 days, a set of 20.9 percent was obtained. No further seed was obtained until the forty-second day, when 1 spike of the 4 that remained in storage had 5 open flowers and set 5 lateral seeds (fig. 2, *C*). The remaining 3 spikes had no open flowers. They presumably had opened and closed. It is worthy of note that the fertile spike mentioned was the only one of the 125 that had had all 6 rows of flowers emasculated.

The productivity of ovules stored at 40° F. decreased with storage age. Thus, ovules stored 29 days produced one seed (fig. 2, *D*), which did not grow. Fresh pollen was not available until the thirty-first day of storage, when, for the first time, no seed was produced.

Storage at 50° F. produced rapid deterioration of the ovule, since but 1 seed set on the fourteenth day out of 93 flowers dusted with pollen stored 3 days (fig. 2, *E*).

Germination tests of seed obtained in both greenhouse and field plot experiments were made on filter paper in Petri dishes at 15° C. for 3 days. Representative seedlings were then transplanted to a greenhouse flat. Table 8 shows the results of these tests, and figure 3 shows the seedlings as they appeared 9 days after transplanting. After 8 weeks of growth in the flat all plants shown in figure 3 except one in row 9 were still living and in spite of the crowding seemed normal. Plants in rows 10, 11, 12, 13, 15, and 16 were shooting or fully headed. Seeds from spikes emasculated or stored or both suffered a marked reduction in weight, but a seemingly normal plant was produced from a seed weighing but 4.6 mg. (row 14, plant 1), as compared with a weight of 35.7 mg. for seeds produced on a normal plant.

TABLE 8.—*Germination of seeds from normal and stored spikes of barley*

Seedling row No. (fig. 3)	Treatment	Storage		Average seed weight	Seeds	Seeds germinated
		Duration	Temperature			
		Days	*° F.*	*Milligrams*	*Number*	*Number*
	GREENHOUSE-GROWN HANNCHEN					
1	Matured on plant			49.5	13	13
2	Emasculated, pollinated, and matured on plant.			22.9	38	38
3	Emasculated, pollinated, excised, and grown in tap water.			12.9	59	59
4	Unemasculated, excised, and grown in tap water.	21	36	8.8	3	1
5	....do	16	40	16.7	9	9
6	Stored pollen on excised emasculated spikes	21	36	19.3	5	5
7	....do	16	40	18.3	4	4
8	Emasculated spikes stored in tap water and pollinated.	21	36	8.8	11	10
9	....do	21	40	7.9	15	14
	FIELD-GROWN BEARDLESS [6]					
10	Matured on plant			35.7	6	5
11	Unemasculated, excised, and grown in tap water.	29	36	23.8	7	6
12	....do	26	40	19.2	1	1
13	....do	14	50	12.4+	10	[1] 1
14	Stored pollen on excised emasculated spikes	26	36	5.1	3	2
15	....do	19	40	7.5	4	1
16	Emasculated spikes stored in tap water and pollinated.	42	36	13.2	5	4
	....do	29	40	2.0	1	0
17	....do	14	50	13.1	1	1

[1] Sample badly moth-eaten.

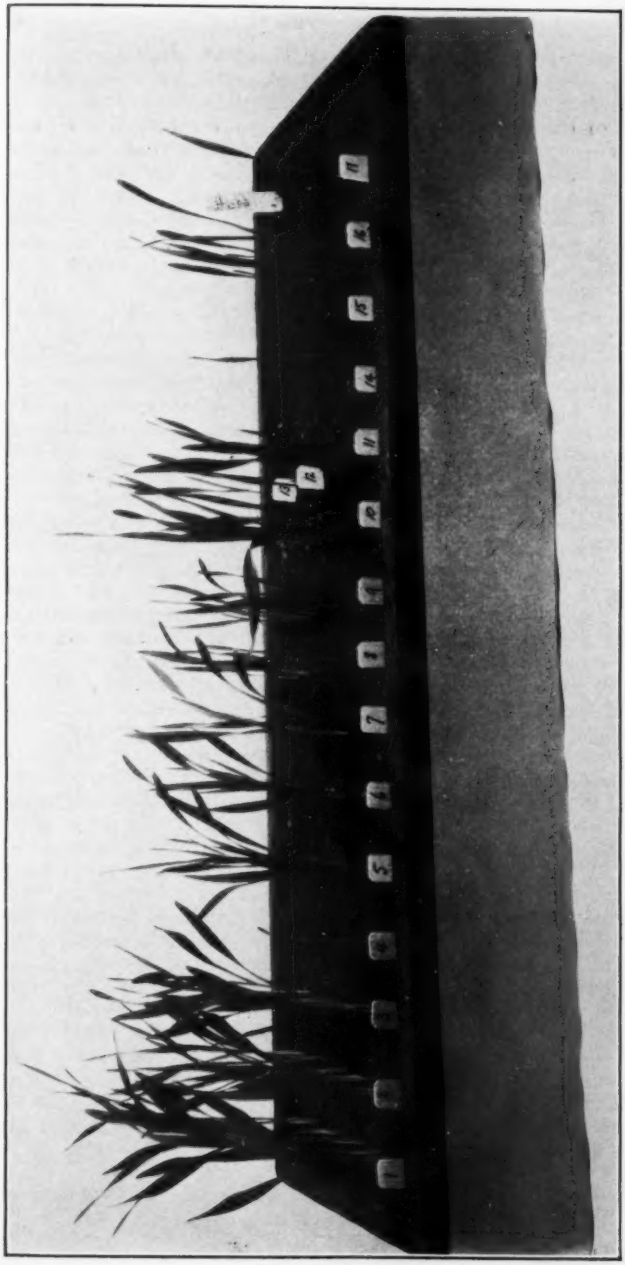

FIGURE 3.—Seedlings 12 days old from seeds produced by pollen and ovules that had undergone cold storage as described in table 8.

DISCUSSION

The spike of barley is treated quite severely in the emasculation process. The boot leaf is cut away just under the spike, the lateral kernels are pulled off, and the lemma and palet are cut off just above the tips of the unextruded anthers, which are removed with forceps. A glassine bag slipped over the spike helps to prevent excessive transpiration. Nevertheless, under good conditions, the seed set in carefully prepared cross-pollinated flowers is high. Thus, during the summer of 1938, at the Aberdeen substation of the Idaho Agricultural Experiment Station, H. V. Harlan and Mary L. Martini [3] emasculated and pollinated a total of 8,085 barley florets, using over 100 varieties as male parents. These florets produced 6,154 seeds, or a set of 76.1 percent. Atlas, the best female parent, gave a 90.5 percent set on 1,402 flowers.

A good set of seed also is obtained when a culm is cut from the plant, the cut end is placed in distilled water, and the flowers are emasculated and pollinated. Four spikes treated in this way in 1934 yielded a set of 81.9 percent.[4] In the present study, with 6 spikes a much lower set (52.7 percent) was obtained. The subjecting of such an excised, emasculated spike to cold storage doubtless increases the hazards of hybridization. Normal growth and development are interrupted, and life processes are greatly slowed down. The flowers of the emasculated spike in cold storage open slowly, remain open for several days, and then close with the stigma hairs beginning to wither at the tips. The anther filaments seldom elongate, and dehiscing anthers are relatively rare. Under such conditions there is much difficulty in getting viable pollen. Whole spikes may die without setting seed, and some flowers set seeds that develop a few days and then stop growing. Notwithstanding these difficulties, two spikes stored 19 days at 36° F. and dusted with pollen stored 8 days at the same temperature set 28 seeds in 36 florets, or 77.8 percent, and one of them set 16 seeds in 19 florets, or 84.2 percent.

The results herein recorded do not show a perfectly orderly decrease of viability in cold storage, as knowledge of the best stage for storage of such material was lacking and a rather wide range of stages was tried. It is possible that for best results emasculation should be done when the anthers are still green but after the spikes have attained considerable stiffness and the glumes have begun to green up. An untried suggestion is that spikes bearing their first-opened flowers be removed from storage at about the time the male parent is beginning to flower, and pollinated when a goodly number of the flowers have opened. Another suggestion is to emasculate all 6 rows of flowers, as a wider range of flower stages on the same spike would be obtained. As has been stated, the one spike to set seed after 42 days of storage was the only one of the 125 that had all 6 rows of flowers emasculated.

This technique makes it possible for plant breeders to obtain viable hybrid seed between early and late varieties of barley seeded at the same time or even between fall-sown and spring-sown sorts.

[3] Oral communication.

[4] POPE, MERRITT N. THE PRODUCTION OF BARLEY SEED BY POST-HARVEST POLLINATION. Jour. Hered. 26: 411–413, illus. 1935.

SUMMARY

Culms of barley with spikes, some emasculated and others unemasculated, were cut from the plant above the third node below the spike, and their cut ends were placed in vessels containing tap water. After cold storage at 36°, 40°, and 50° F. for various periods, samples were removed and their pollen and ovules tested as to their ability to produce seed.

Spikes excised and placed in cold storage within 3 days after emasculation had the highest set of seed after they were returned to the greenhouse.

Growth in length of culm practically ceased after 1 to 2 days in cold storage.

The elongation of the anther filament and the dehiscence of the anther were largely prevented in dark cold storage.

Flower opening and closing in emasculated spikes proceeded at a greatly reduced speed in cold storage, the rate being slowest at the lowest temperature.

Color in the culm was well preserved at 36° and less well preserved at 40° F. The culms stored at 50° were quite yellow on the nineteenth day and chlorotic on the twenty-sixth day.

Unemasculated spikes set seed after 29 days of storage at 36° F., after 26 days at 40°, and after 14 days at 50°.

The extreme limit of storage for productive pollen was found to be 26 days at 36° F. and 19 days at 40° F. No pollen stored for 14 days or longer at 50° produced seed.

The extreme limit of storage found for production of seed from stored emasculated spikes was at least 42 days at 36° F., 29 days at 40°, and 14 days at 50°.

Seed from all the foregoing tests, except the single small seed produced on the emasculated spike stored for 29 days at 40° F., germinated and gave apparently normal plants after 8 weeks of growth.

Cold storage of undisturbed pollen or preferably of excised, emasculated spikes of barley makes it possible to obtain viable hybrid seed between varieties that normally flower on rather widely separated dates.

EFFECTIVENESS OF HEAT PENETRATION IN THE CANNING OF MEAT IN THE HOME BY THE PRESSURE COOKER [1]

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INTRODUCTION

In a study of the effectiveness of heat penetration in meat canning sterility must be considered from two points of view: (1) Sterility in the strict laboratory sense, which implies absolute absence of all germ life. Numerous investigations show that there is a probability that this goal is seldom attained in canning meats. (2) Commercial sterility, which concerns those meats that keep and are safe for use over fairly long periods of storage. These products are termed sterile in a commercial sense.

It is conceivable that under the conditions of commercial sterility there are several possibilities: (1) Canned products which might not, be sterile in the strict laboratory sense but would keep in storage for a reasonable length of time; (2) conditions in which the organisms might still be present but only in a dormant stage; (3) conditions such that should the bacteria grow, they would be capable of causing spoilage only, and, not forming toxic substances, would offer no danger to the consumer.

When a whole carcass is to be canned and the many different parts are to be preserved, it is obviously necessary to alter the processing and packing to fit the cut and type of pack. Meat is a very poor conductor of heat, but convection currents in a fluid pack may permit a more rapid elevation of the temperature than in a solid type of pack. Clear juice or broth alone without the meat permits a more rapid circulation of heat in the container than when meat is present to interfere with the circulation. With these facts in mind and in order to make the results representative, the writers used various packs of meat such as are commonly employed in the home, and in exact duplicate.

The investigations of the factors controlling heat penetration were made for the purpose of determining their effect (1) in eliminating all dangerous organisms and (2) in controlling spoilage by the more persistent non-disease-producing organisms that are commonly found in canned meats.

EXPERIMENTAL PROCEDURE

In order to determine accurately, the internal temperature during the processing period, a 12-quart home type of pressure cooker similar to one described and illustrated by Magoon and Culpepper was used.[2] The equipment permits a long-stemmed thermometer to

[1] Received for publication February 24, 1939. This project was originally sponsored by the Federal Emergency Relief Administration following the drought years of 1934 and 1935 when stock breeders of the Dakotas and Montana had to dispose of their extensive herds because of the feed shortage. The writers herein acknowledge their gratitude and appreciation to the directors of the Federal Emergency Relief Administration for their valuable assistance in this work.

[2] MAGOON, C. A., and CULPEPPER, C. W. A STUDY OF THE FACTORS AFFECTING TEMPERATURE CHANGES IN THE CONTAINER DURING THE CANNING OF FRUITS AND VEGETABLES. U. S. Dept. Agr. Bul. 956, 55 pp., illus. 1931.

be inserted through the cover of the retort, directly through a brass plate soldered to the cover of the can, and into the center of the contents. The temperature can be read constantly and appropriate records made.

A group of organisms such as are most likely to occur in canned meats was chosen to test the effectiveness of the sterilizing process. This group consisted of *Clostridium botulinum*, *Escherichia coli*, a heat-resistant strain of *Streptococcus faecalis* isolated from a can of spoiled meat, and *Bacillus mesentericus*. *Cl. botulinum* (both strains A and B) was grown in a well-buffered proteose-peptone broth and aged 5 to 6 weeks, until the culture was almost entirely in the spore stage. It is well known that freshly-formed spores of *Cl. botulinum* possess little more resistance to heat than vegetative forms. Well-aged spore cultures were therefore required to represent the heat-resistant spore forms that are present in the soil and dust.

Escherichia coli is representative of the great group of non-spore-forming organisms found in the intestines of animals and is constantly associated with the organisms of infectious food poisoning. *E. coli*, being more generally resistant to conditions unfavorable to bacterial development, is a fair indicator for the survival of the pathogenic *Salmonella* varieties and other such food poisoners of animal intestinal origin.

From several cans of spoiled meat previously examined, there had been isolated a Gram-positive streptococcus which is quite heat resistant. Moist heat at 75° C. for 10 minutes is required to kill it. Cultural characteristics indicate its relationship to *Streptococcus faecalis*.[3] All evidence indicated that this particular strain had appeared in the spoiled meat as a result of inadequate washing of carcasses at the time of slaughter and dressing. This streptococcus is frequently associated with *Escherichia coli* in meat spoilage, and, while not a gas producer, contributes to the fermentation of canned meat even under anaerobic conditions.

Bacillus mesentericus has been reported frequently in the spoilage of canned vegetables and meat where leaky cans are involved. The fact that it possesses a very heat-resistant spore suggested the use of this strict aerobe as an indicator of heat efficiency.

Various methods of introducing the organisms into the material to be canned were used. *Bacillus mesentericus* spores were dried on bits of sterile gauze which were placed in a pair of telescoping small test tubes. The disadvantage of this method is that the double walls of glass interfere with ready heat penetration and give high survival results. For this reason a suspension of spores in 1-percent saline in thin-walled, sealed glass ampoules was used. Such a device made it possible to place the organisms in a central position in the can where they could easily be found again. Suspensions of spores or vegetative cells in 0.5 cc. amounts were also injected directly into the body of the meat by hypodermic needle. Recovery of cultures was attempted from one of the duplicate cans immediately at the end of the process, and again from the contents of the second can at the end of the storage period. Meat, juice, and the contents of the ampoules were each tested separately with appropriate media under conditions of

[3] This streptococcus grows well on ordinary media. It occurs in chains of three or four cocci- is Gram-positive, nonhemolytic, and produces a small amount of "greening" on fresh blood agar. It produces acid in lactose, levulose, mannite, salicin, saccharose, raffinose, and inulin. A facultative anaerobe and *Escherichia coli* were found associated with it in several cans of meat.

anaerobiosis, aerobiosis, or in carbohydrate media chosen to make quick identification possible.

Duplicate cans were stored for 2 to 5 months, which represents the average time of storage in the home. The temperature was maintained at about 35° C. This time and temperature represent conditions under which it is possible for spores of *Clostridium botulinum* to recover from the shock of heating and germinate. Delayed germination of spores is a possible danger. Dickson and his coworkers [4] concluded that the spores of *Cl. botulinum* may remain dormant as long as 37 months after exposure to heat, and then germinate, multiply, and produce toxin. It has been shown recently [5] that germination of spores of *Cl. botulinum* is inhibited at temperatures of 0° to 15° but is hastened at 30° to 35°. However, in no instance in the experiments reported here was growth of *Cl. botulinum* obtained from a stored can when it was not obtained from the first can also. In one case involving spores of *Bacillus mesentericus* a growth was obtained from a stored duplicate can when it was not obtained from the original one. In general, however, the duplicate cans checked perfectly.

The top cut of a round of beef was selected for use in the comparative canning tests because it consists of a tougher muscle which is usually preserved by canning methods, and also because it lends itself well to sectioning. The eye muscle of the round, when wrapped in a single layer of cheesecloth is easily located in the pack after processing and is large enough to allow one to place several prepared ampules in it as well as to inoculate it with a long hypodermic needle.

In packing the cans of meat, it was found that by having the top chuck cut into a slice three-fourths of an inch thick and again sectioned into pieces approximately 2½ inches square each pack was readily duplicated. This method also permitted the insertion of the thermometer into an identically placed piece of meat each time and obviated the possibility of the thermometer bulb recording liquid or space temperature rather than the temperature of the meat.

A number of packs varying in weight and size were processed. It was estimated that 495 gm. for No. 2, 725 gm. for No. 2½, and 885 gm. for No. 3 cans represented packs that allowed for sufficient head space in the can and gave the most desirable results. Three or more cans were used for each test of variation.

In order to allow for variation in initial temperatures and have all packs processed under as nearly identical conditions as possible, duplicate cans filled with the same weight of meat were preheated in a bath of boiling water which came to within 1 inch of the top of the cans. All cans were preheated until their contents registered 31° C. This rise in temperature usually took 1 to 40 minutes, depending on the size, weight, and nature of the contents as well as the temperature at which the meat was packed.

Duplicate cans were then sealed and the thermometer was inserted in the center of the can attached to the cover of the retort. One inch of boiling water was placed in the retort, the duplicate can was placed on the bottom of the cooker, the cover adjusted, and the apparatus sealed. After the retort was sealed the pet cock was left open for

[4] DICKSON, ERNEST C., BURKE, GEORGINA S., BECK, DOROTHY, and JOHNSTON, JEAN. STUDIES ON THE THERMAL DEATH TIME OF SPORES OF CLOSTRIDIUM BOTULIUM. IV. THE RESISTANCE OF SPORES TO HEAT AND THE DORMANCY OR DELAYED GERMINATION OF SPORES WHICH HAVE BEEN SUBJECTED TO HEAT. Jour. Infec. Diseases of 36; [472]–483. 1925.

[5] TANNER, FRED W., and OGLESBY, ELAINE E. INFLUENCE OF TEMPERATURE ON GROWTH AND TOXIN PRODUCTION BY CLOSTRIDIUM BOTULINUM. Food Res. 1: 481–494. 1936.

7 minutes to allow the enclosed air to escape. The pet cock was then closed and the temperature of the meat was recorded at 1-minute intervals. The processing time was calculated from the time the retort attained the desired pressure. The retort was then removed from the heating element, the pet cock opened, and the steam fully released. The bulged cans were then cooled by one of two methods: (1) By being allowed to remain at room temperature until quite cool, or (2) by being plunged into cold running tap water at approximately 10° C.

Examination of the contents of the can in which the thermometer had been placed was made on the day of canning, the duplicate can being stored. At the end of the storage period, the contents were again checked for keeping quality and for evidence of bacterial growth or survival.

EXPERIMENTAL DATA

Graphs were prepared from the temperature readings made as described. So far as possible only one variable at a time was permitted in these experiments. The chief variables were: (1) Type of pack; (2) steam pressure (i. e., 10 pounds and 15 pounds); (3) can size; (4) duration of process; and (5) methods of cooling (i. e., cold dipping and room-temperature cooling). While the size of the apparatus permitted only two cans at a time to be processed each variation in the experiments was repeated three or more times to make sure that results were capable of duplication. The data closest to the average for the series of trials were used in the following graphs.

For comparison, the maximum temperature attainable under 10 pounds and 15 pounds of steam pressure is indicated by heavy horizontal lines. This is the ceiling or upper limit of temperature attainable in the can at the pressure used. The crest of the curve entering the zone between 100° C. and the ceiling defines the dome of actual sterilization, a product of temperature and time. The area of the dome within this zone marks the actual temperature-time-sterilization in the particular experimental case. The efficiency (i. e., effective heat penetration) of any arrangement of the equipment is expressed as a percentage upon each curve. This percentage is the ratio of the area within the dome to that of the theoretical sterilizing zone within the time limits.

Bacteria survival tests at different temperatures are also imposed upon the curves to indicate the probable effectiveness of the process. These results may or may not be consistent with the percentage results.

In figure 1 it may be seen that heat penetration is readiest where the least interference with convection currents is met, that is, in the order: (1) Liquid; (2) ground-meat patties; (3) raw cubed beef; and (4) raw ground meat, solid pack. Though the nutrient broth was processed at only 10 pounds pressure, it heated so effectively that sterilization was accomplished, which was not true of all the other packs at a higher steam pressure.

Under 15 pounds pressure absolute sterility was not assured, but the ever dangerous *Clostridium botulinum* and the intestinal organism *Escherichia coli* were destroyed even when the efficiency ratios were low. The surviving *Bacillus mesentericus* is a strict aerobic organism,

and cannot grow in a perfectly sealed can. In "leakers" it can grow and cause very apparent spoilage. It is exceedingly difficult to eliminate the spores of this soil organism.

Two curves representing identical cans, but with one at 15 pounds pressure and the other at 10 pounds pressure, show a wide difference in heat penetration (fig. 2). The difference is sufficient to account for differences in bacterial results.

Heat penetration as influenced by variation in can size is shown in figure 3; no effort was made to represent any variation in can area by the use of cans of different shapes. A close correlation between heat penetration and can size is indicated. As in figure 1, *Bacillus mesentericus* spores survived but other organisms were killed.

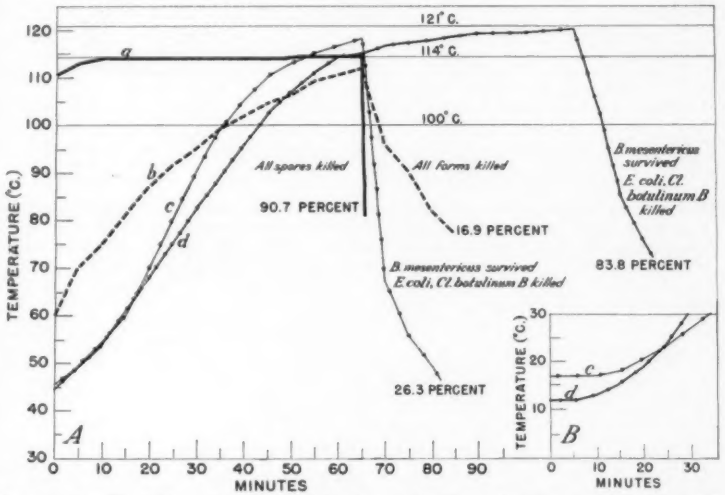

FIGURE 1.—Variations in effective heat penetration, shown as percentage (see text for fuller explanation), caused by type of pack: *A*, Heating period; *B*, preheating period: *a*, 725 cc. of nutrient broth processed at 10 pounds pressure in No. 3 can; *b*, 725 gm. of pan-fried beef patties processed at 15 pounds pressure in No. 3 can; *c*, 885 gm. of raw cubed beef processed at 15 pounds pressure in No. 3 can; *d*, 885 gm. of raw ground beef processed at 15 pounds pressure in No. 3 can.

In figure 4, which shows the time variable, maximum heat penetration is seen to have been reached at 90 minutes. This series of trials was run to determine the feasibility of continuing the process until actual sterility was attained. Actual sterility was attained in 90 minutes. As shown in the other figures, *Escherichia coli* and *Clostridium botulinum* spores do not survive the 60-minute period in No. 2½ cans at 15 pounds pressure. This, then, indicates a threshold of safety at 65 minutes under these conditions of can size and pressure. It indicates also the possibility of actual sterility at 90 minutes, but the necessity is not shown.

The fact is self-evident that cooling at room temperature prolongs the sterilization process within the can. During the cooling process

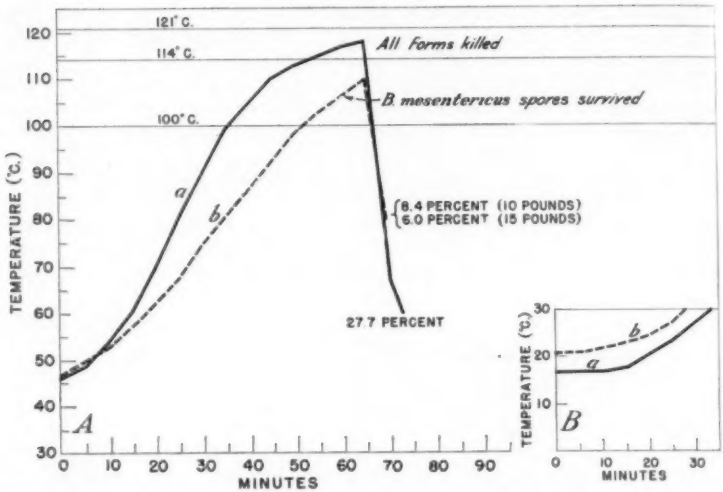

FIGURE 2.—Variations in effective heat penetration, shown as percentage (see text for fuller explanation), caused by differences in steam pressure while processing: *A*, Heating period; *B*, preheating period; 725 gm. of raw cubed beef processed at 15 (*a*) and at 10 (*b*) pounds pressure for 65 minutes in No. 2½ cans.

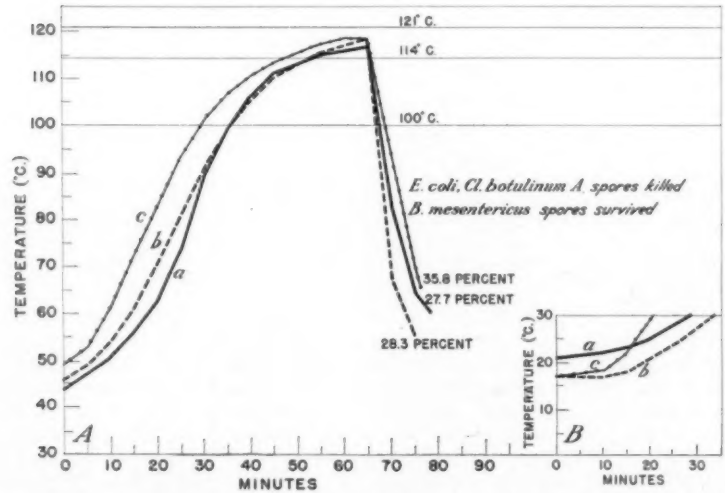

FIGURE 3.—Variations in effective heat penetration, shown as percentage (see text for fuller explanation), caused by differences in can size in processing raw cubed beef at 15 pounds pressure for 65 minutes: *A*, Heating period; *B*, preheating period: *a*, 885 gm. in a No. 3 can; *b*, 725 gm. in a No. 2½ can; *c*, 495 gm. in a No. 2 can.

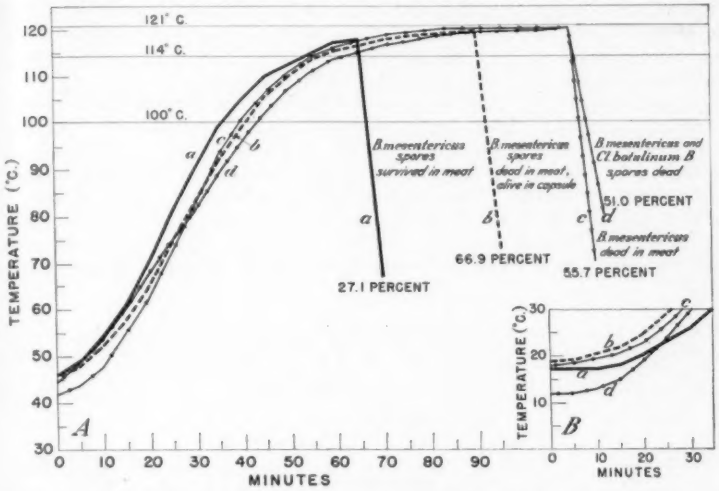

FIGURE 4.—Variations in effective heat penetration, shown as percentage (see text for fuller explanation), caused by differences in time of heating at 15 pounds pressure in No. 2½ cans: *A*, Heating period; *B*, preheating period: *a*, 725 gm. of raw cubed beef containing *Bacillus mesentericus* heated for 65 minutes; *b*, 725 gm. of raw cubed beef containing *B. mesentericus* heated for 90 minutes; *c*, 725 gm. of raw cubed beef containing *B. mesentericus* heated for 110 minutes; *d*, 725 gm. of raw cubed beef containing *B. mesentericus* and *Clostridium botulinum*, B strain, heated for 110 minutes.

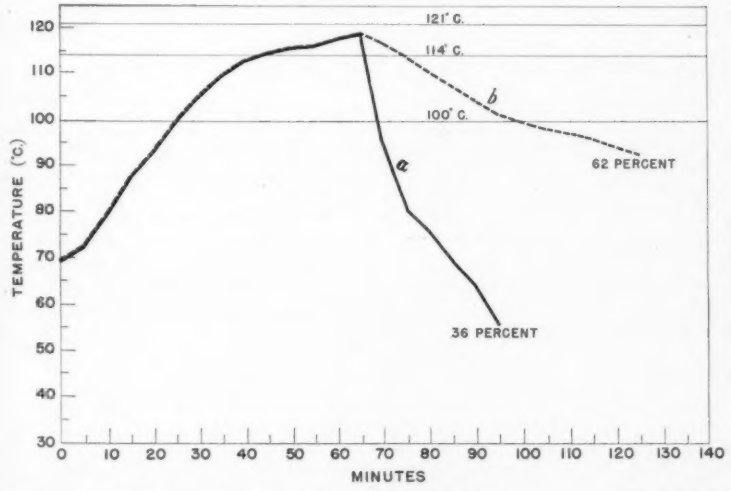

FIGURE 5.—Variations in effective heat penetration, shown as percentage (see text for fuller explanation), as related to method of cooling: 725 gm. of raw cubed beef processed for 65 minutes at 15 pounds pressure in a No. 2½ can (*a*) cold-dipped and (*b*) left at room temperature.

cooking also progresses (fig. 5). The use of room-temperature cooling may well make it feasible to decrease the period of processing or the pressure needed to pass the threshold of safety. Bacterial test results were consistent with the percentage efficiency.

In addition to the five variables represented in figures 1 to 5 there is another factor which has proved important in the sterilization of meat in the can. This is the temperature of the meat upon its receipt as modified by the preheating period. As shown in table 1, the temperature increase during the preheating period is very slight during the first 5 minutes but accelerates constantly in the following 5-minute periods. If the preheating period is extended to 20 minutes or longer, the temperature difference between meats of different temperatures is eliminated.

TABLE 1.—*Temperature rise in successive 5 minutes of the preheating period of canned round of beef, later processed for 65 minutes at 15 pounds pressure, as related to initial temperature of the meat*

Process[1]	Initial temperature	Temperature at the end of—				Length of processing	Internal temperature on removal from can	Comments
		5 minutes	10 minutes	15 minutes	20 minutes			
	°C.	°C.	°C.	°C.	°C.	*Minutes*	°C.	
Steamer-exhausted	8	9	12	20	29¾	65	95	1 inch of water in steamer for exhausting.
Pan-seared	8	------	------	------	------	65	99	Internal temperature 57°-65° C. when packed into cans; 94° C. when cover was removed.
Seared in oven; preheated to 500° F.	10	14	16	22	23 24 30	65	98	93° C. when cover was removed (salt omitted).
No exhausting; packed cold	10	------	------	------	------	65	98¾	Expansion sounds at 15 minutes; 91° C. on opening.
Surplus meat exhausted 20 minutes; water 1 inch from top of cans	10	16	19	25	30 33	55	97	Length of time cut 10 minutes; more water in steamer for exhausting.

[1] 24 pounds of beef round as received. Meat wiped with damp cloth, sliced approximately ¾ inch thick and into pieces 2½ inches square; ½ teaspoon of salt added to each can. No. 2 cans used. Average weight when packed 1¼ pounds. 3 cans used in each test: 1 can plunged into cold water immediately upon removal from pressure cooker; 1 can opened immediately and internal temperature taken; 1 can left at room temperature without cooling. On opening ,all meat seemed stringy and appeared overcooked.

Table 2 reports the same type of experiments with beef received frozen (average temperature −2° C.) and placed in cans only after it had thawed out. During the fourth 5-minute interval of preheating the temperatures reached were not so uniform as those in table 1. This would seem to imply that the differences would be eliminated in still later 5-minute periods.

TABLE 2.—*Temperature rise in successive 5 minutes of the preheating period of canned sirloin of beef, received frozen, and steamer-processed*[1] *at 15 pounds pressure for different periods of time*

Initial temperature ° C	Temperature at the end of—				Length of processing	Time for gage to reach 15 pounds	Internal temperature on removal	Comments
	5 minutes	10 minutes	15 minutes	20 minutes				
	° C.	° C.	° C.	° C.	*Minutes*	*Minutes*	° C.	
6	6	12	23	31	45	3	97	Expansion sounds at 10, 16, 21 minutes; 2 at 26 minutes; 95° C. on opening.
7	7	9	18	23				
7	7	7	12	17				
1	1	1	1	7	40	28⅝₀	98	Expansion sounds at 6, 11, 14, 21, 33 minutes; 90° C. on opening.
0	0	1	3	11				
0	0	3	7	14				
8	8	12	20	29	30	3	96	Expansion sounds at 10, 11 minutes; 2 at 13, 14, and 14½ minutes; 91° C. on opening.
4	4	13	14	17				
9	11	18	25	32				
11	15	17	20	25				
13	14	20	27	34	20	3	84	Expansion sounds at 2, 3, 14 minutes; 4 minutes, and 20 minutes, as pressure was being released.
10	10	12	17	23				

[1] 17½ pounds sirloin of beef received frozen, average temperature −2° C. Left at room temperature until approximately 0° C. Meat wiped with damp cloth and sliced ¾ by 2½ by 2½ inches. 1¼ pounds total weight of packed No. 2 cans. All cans exhausted 20 minutes in 1 inch of boiling water. Thermometers inserted into center of meat and temperatures taken each 5-minute period of exhausting. 3 cans used in each test: 1 can opened immediately on removal from pressure cooker; 1 can dipped into cold water until ends contracted; 1 can left at room temperature. All cans labeled and stored at room temperature.

From these observations, it appeared that the setting of an arbitrary period for preheating was not sufficiently accurate for this type of work. Therefore, in all the experiments reported the steam processing began only when the preheating had produced a temperature of 31° C.

SUMMARY AND CONCLUSIONS

In solid meat packs where convection currents are not possible, temperatures reached under 10 pounds of steam are insufficient to destroy such bacteria as were tested in this experiment. Sixty-five minutes at 15 pounds pressure (121° C.) appeared to be the minimum time and temperature that could safely be allowed for sterilization of No. 2 cans, 90 minutes for No. 2½ cans, and 110 minutes for No. 3 cans.

Cold dipping proved to be the most desirable means of treating the cans after they had been removed from the retort. This process reduces the cooking temperature at once and so eliminates the danger of overcooking and at the same time reduces the pressure exerted on the seal. Leaving the processed cans at room temperature prolongs the already long cooking period, gives an overcooked product, and because of the unrelieved internal steam pressure, strains the seal.

Clostridium botulinum spores of both strains A and B were destroyed even when the heat efficiency was low. When the processing time was extended over a period of at least 65 minutes at 15 pounds pressure the elimination of food poisoning organisms was certain. It was found, however, that the temperatures attained at even the higher pressure level were not uniformly effective in destroying thermoduric organisms such as *Bacillus mesentericus* and *Streptococcus faecalis*.

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Heat penetration is readiest where the least interference with convection currents is met, in the order: (1) Liquid; (2) chunk meat; (3) ground-meat patties; (4) ground meat, solid pack; and (5) solid chunk. The probability exists that certain heat-resistant, putrefactive aerobes will survive the practical limit of prolonged processing beyond which the product deteriorates. This may also be true of putrefactive spore-bearing anaerobes.

Because of the lag in the heat curves, the ratio of efficiency is surprisingly low. The gradient of the noneffective part of the curve varies in the order of the packs just given.

So far as the experiments have been carried, 15 pounds pressure is indicated as necessary for sterility in the laboratory sense. Within that pressure limit the time variable should be made to suit the type of pack and size of container. In the canning of beef in tins where 15 pounds steam pressure is used over periods of time dependent upon the variation in procedure, the threshold of safety has been reached and probably passed. The term "safety" as here used refers to freedom from food poisoning by *Clostridium botulinum* and to infectious food poisoning by the *Salmonella* group and allied intestinal organisms. By careful control of still other factors which influence rapidity of heat distribution in the can, such as fluid and fat, it would undoubtedly be possible to process meat just under the threshold of safety and still have a product that would keep for a reasonable length of time.

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